自主食用不同甜味物质对小鼠尿液蛋白质组的影响

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摘要

目的：通过分析小鼠自主食用不同甜味物质后尿液蛋白质组的变化，探索其对机体的可能影响。

方法：收集 C57BL/6l 小鼠主动食用甜味物质前后的尿液样品。甜味物质包括目前世界范围内应用较为广泛且能引起小鼠喜好反应的蔗糖、甜菊糖苷、安赛蜜、三氯蔗糖，其中非营养性甜味剂的浓度选择了已有研究显示的小鼠喜好反应最强烈的浓度。通过高效液相色谱串联质谱联用（LC-MS/MS）的非标记定量蛋白质组学技术进行分析，成组筛选尿液蛋白质组的差异蛋白，进行蛋白质功能和生物学过程分析；进行单只小鼠食用甜味物质前后尿液蛋白质组比较；并将不同甜味物质进行横向对比。

结果及结论：尿液蛋白质组可以反映小鼠自主食用甜味物质后的机体变化，且小鼠自主食用的不同甜味物质对尿液蛋白质组的影响并不相同。4 种甜味物质中，三氯蔗糖与蔗糖引起机体的变化最为相似，甜菊糖苷与蔗糖引起机体的变化相差最远；蔗糖、安赛蜜和三氯蔗糖引起的机体变化相似，而甜菊糖苷引起机体的变化与其他甜味物质不同。小鼠自主食用 4 种甜味物质后尿液蛋白质组差异蛋白中均有已经被报道的与脑奖励回路相关的蛋白，而只有自主食用蔗糖、安赛蜜和三氯蔗糖后尿液蛋白质组差异蛋白大量与代谢过程相关，自主食用甜菊糖苷后尿液蛋白质组差异蛋白主要与核小体组装、基因表达等过程相关。

关键词：尿液蛋白质组 甜味物质 糖代谢 脑奖励通路

Effects of self-consumption of different sweet substances on urine proteome of mice

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Abstract

Objective: To explore the possible effects of different sweet taste substances on the body by analyzing the changes of urinary proteome in mice after self-consumption of different sweet taste substances.

Methods: Urine samples of C57BL/6l mice were collected before and after self-consumption of sweet substances, including sucrose, stevia glycosides, acesulfame and sucralose, which are widely used in the world and can cause the preference reaction of mice. Among them, the concentration of non-nutritive sweeteners was selected as the concentration that has been shown to have the strongest preference reaction of mice. Label-free quantitative proteomics using high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used for

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analysis. Differential proteins of urine proteome were screened in groups for analysis of protein functions and biological pathways. The urine proteome of single mice before and after self-consumption of sweet substances was compared, and the common differential proteins were counted; and the different sweet substances were compared horizontally.
Results and conclusions: Urine proteome can reflect the body changes of mice after self-consumption of sweet substances. And the effects of different sweet substances on urine proteome were different. Among the four sweet substances, sucralose caused the most similar changes in the body compared with sucrose. Compared with sucrose, stevia glycosides caused the most different changes in the body. The body changes caused by sucrose, acesulfame and sucralose are similar, but the body changes caused by stevia glycoside are different from other sweet substances. After self-consumption of the four sweet substances, the urine proteome differential proteins in mice all had proteins that had been reported to be related to brain reward circuitry, while only the urine proteome differential proteins after self-consumption of sucrose, acesulfame and sucralose were mainly related to metabolic processes. Urine proteomic differential proteins after acesulfame of stevia glycoside were mainly related to nucleosome assembly and gene expression.
Keywords: urine proteome; sweet substances; glucose metabolism; brain reward pathway

1 引言

糖作为碳水化物的一种，可以分为单糖：葡萄糖、果糖和半乳糖；双糖：蔗糖（Sucrose）、乳糖和麦芽糖 [1]。糖直到近代才走向大多数人的饮食中，成为了寻常的调味品，且能够提供大量的能量和令人愉快的甜味觉体验，但是人类祖先是在低糖的环境下进化而来，很难在短时间内适应糖在饮食中的突然大量添加[2 3]。越来越多的研究表明，过量摄入糖会导致肥胖[4]、糖尿病[5]、心血管疾病[6]、非酒精性脂肪肝[7]、龋齿[8]和某些癌症[9]的风险增加；同时由于人类对甜味先天的敏感，导致不能短时间适应高浓度的甜味刺激，过量摄入糖，会在脑中产生超越正常的脑奖励信号，可能覆盖自我控制机制，导致成瘾，强烈的甜味觉刺激可以超过可卡因刺激的奖励[2 3]。

越来越多的人意识到了摄入过量糖的风险，而非营养性甜味剂作为糖的替代品，以其不含或低热量，且能提供更强烈的甜味觉体验而越来越受欢迎，广泛用于食品和饮料等产品的生产中[10]。目前世界范围内应用最为广泛的非营养性甜味剂主要包括阿斯巴甜、安赛蜜（Acesulfame）、三氯蔗糖（Sucralose）、甜菊糖苷（Stevia Glycosides）等[11]。但是对于非营养性甜味剂的安全性和有效性一直存在争议。为期 11 年的研究表明，每月饮用 2 份及以上含非营养性甜味剂的饮料会增加患冠心病和慢性肾病的风险[12 13]，在一项对 17 名被试者的研究中，摄入三氯蔗糖相对于摄入水后的糖耐量实验中，血浆葡萄糖峰值浓度增加、胰岛素分泌率增加 20%[14]。面对日益增长的非营养性甜味剂的使用，重新进行有效的大规模的评估，筛选安全性高、更受大众喜欢的非营养性甜味剂是急需解决的问题。

尿液是血液经肾脏过滤所产生，用以排除代谢废物，不受内环境稳态调节机制的控制，能更敏感地保留机体产生的各种微小变化[15]。已有研究提出，通过尿液代谢组寻找生物标志物，可用于提供评估非营养性甜味剂的客观摄入，从而加强基于人群的研究[16]。但是尚未有研究通过尿液蛋白质组对甜味物质，包括糖和非营养性甜味剂对机体的影响进行研究。相对于尿液代谢组，尿液蛋白质组的变化则能直接反映出机体的变化，加之其本身的灵敏性，具有直接进行糖和非性甜营养味剂对机体短期和长期影响的研究的潜力。本研究目前世界范围内应用较为广泛且能引起小鼠喜好反映的甜味物质：蔗糖、甜菊糖苷、安赛蜜、三氯蔗糖，其中非营养性甜味剂浓度均选择了已有研究显示的小鼠喜好反应最强

烈的浓度[17 18]，通过收集小鼠主动食用不同的甜味物质后前的尿液样本，对尿液蛋白质组进行比较研究。尝试探索糖和非营养性甜味剂导致的机体变化是否能够在尿液蛋白质组中反映，进而探究哪种非营养性甜味剂对机体的影响较小、和蔗糖的影响接近，非营养性甜味剂是否存在对机体的其他潜在影响。

2 实验方法

2.1 实验动物

10 周龄 C57BL/6l 雄性小鼠 9 只，购于北京维通利华实验动物生物技术有限公司。所有小鼠在标准环境中饲养（室温（22±1）℃，湿度 65%-70%）。将所有小鼠在新环境中饲养三天后开始实验，一切实验操作遵循北京师范大学生命科学学院伦理委员会的审查和批准，批准编号为 CLS-AWEC-B-2022-003。

2.2 尿液样本收集

本研究选择的甜味物质是目前世界范围内应用较为广泛且能引起小鼠喜好反映的蔗糖、甜菊糖苷、安赛蜜、三氯蔗糖，非营养性甜味剂浓度均选择了已有研究显示的小鼠喜好反应最强烈的浓度[17 18]。

2.2.1 蔗糖组尿液样本收集

4 只雄鼠禁水 10 h 后，给水 1 h，禁水收尿 12 h，获得蔗糖对照组样本；雄鼠适应性喂养 2 天；雄鼠禁水 10 h 后，舔食 0.2 g 蔗糖粉末，给水 1 h，禁水收尿 12 h，获得蔗糖实验组样本，样本暂存于-80℃冰箱。

2.2.2 甜菊糖苷组尿液样本收集

5 只雄鼠禁水 10 h 后，给水收尿 12 h，获得甜菊糖苷对照组样本；雄鼠适应性喂养 1 天；雄鼠禁水 10 h 后，给 0.3%甜菊糖苷水收尿 12 h，获得甜菊糖苷实验组样本，样本暂存于-80℃冰箱。雄鼠适应性喂养 2 天。

2.2.3 安赛蜜组尿液样本收集

5 只雄鼠禁水 10 h 后，给水收尿 12 h，获得安赛蜜对照组样本；雄鼠适应性喂养 1 天；雄鼠禁水 10 h 后，给 10 mM 安赛蜜水收尿 12 h，获得安赛蜜实验组样本，样本暂存于-80℃冰箱。雄鼠适应性喂养 2 天。

2.2.4 三氯蔗糖组尿液样本收集

5 只雄鼠禁水 10 h 后，给水收尿 12 h，获得三氯蔗糖对照组样本；雄鼠适应性喂养 1 天；雄鼠禁水 10 h 后，给 10 mM 三氯蔗糖水收尿 12 h，获得三氯蔗糖实验组样本，样本暂存于-80℃冰箱。

2.3 尿液样本处理

尿蛋白提取：-80℃冰箱中取出小鼠尿液样本， 4°C 的条件下解冻。4℃，12000×g 离心 30 min，取 2 mL 上清液，每 500 μL 上清液于 2 mL 离心管中，加入三倍体积的预冷无水乙醇，上下颠倒轻柔混匀，-20°C 沉淀过夜蛋白。过夜沉淀的混合液 4°C，12000×g 离心 30 min，弃上清，等待乙醇挥发干燥。将蛋白沉淀重悬于裂解液中(含 8 mol/L 尿素，2 mol/L 硫脲，25 mmol/L 二硫苏糖醇，50 mmol/L Tris)。 4°C，12000×g 离心 30

min，取上清于新的 1.5 mL 离心管内，获得尿液蛋白质。用 Bradford 法测定蛋白质浓度。

尿蛋白酶切：取 100 μg 尿液蛋白质样品于 1.5 mL 离心管中，加入 25 mmol/L NH4HCO3 溶液使总体积为 200 μL。加入 20 mM 二硫苏糖醇溶液（Dithiothreitol, DTT, Sigma），涡旋混匀，金属浴 37°C 加热 1 h，冷却至室温。加入 50 mM 碘乙酰胺（Iodoacetamide, IAA, Sigma），涡旋混匀，室温避光反应 40 min。取 10 kDa 超滤管(Pall, Port Washington, NY, USA) 向滤膜上加入 200 μL UA 溶液 (8 mol/L 尿素，0.1 mol/L Tris-HCl，pH 8.5)洗涤滤膜，18°C，14000×g 离心 5 min，弃去下层滤液，重复一次；向滤膜上加入碘乙酰胺处理完成后的尿液蛋白质样品，18℃，14000×g 离心 30 min，弃去下层滤液，尿液蛋白质留在滤膜上；向滤膜中加入 200 μL UA 溶液洗涤尿液蛋白质，18°C，14000×g 离心 30 min，重复两次；向滤膜中加入 25 mmol/L NH4HCO3 溶液洗涤尿液蛋白质，18°C，14000×g 离心 30 min，重复两次；按胰酶：蛋白为 1：50 的比例加入胰蛋白酶（Trypsin Gold, Promega, Fitchburg, WI, USA）进行酶切，37°C 水浴 15 h。酶切结束后 4℃，13000×g 离心 30 min 收集滤液，该滤液为多肽混合液。将多肽混合液通过 HLB 固相萃取柱(Waters，Milford，MA)进行除盐，使用真空干燥仪冻干，于-20°C 条件下保存。

2. 4 LC-MS/MS 串联质谱分析

0.1%甲酸溶解多肽混合液冻干，使用 BCA 试剂盒对肽段进行定量，用 0.1%甲酸将肽段浓度稀释为 0.5 μg/μL。从每个样品中取 6 μL 混匀，制备混合肽段样本。使用高 pH 反相肽分离试剂盒(Thermo Fisher Scientific)进行混合肽段样本分离。离心收集 10 份流出液（Fractions），使用真空干燥仪冻干，用 0.1%甲酸复溶。10 份流出液和全部单个样品以样品 :iRT 体积比为 10 : 1 的例加入 iRT 试剂(Biognosys, Switzerland)，以校准提取的肽峰的保留时间。

为了进行分析，将来自 10 份流出液和单个样品的 1μg 肽，使用 EASY-nLC1200 色谱系统(Thermo Fisher Scientific, USA)进行分离，分离的肽段经过 Orbitrap Fusion Lumos Tribrid 质谱仪（Thermo Fisher Scientific，Waltham，MA，USA）分析。

为了生成谱库，对 10 份流出液以 Data Dependent Acquisition(DDA)模式进行质谱分析在高灵敏度模式下采集数据，在 350-1200m/z 范围内获得完整的扫描图谱，分辨率设置为 60000。生成 10 份 raw 文件，导入 Proteome Discoverer 软件采用 Swiss-iRT 和 Uniprot-Rat 数据库进行建库分析（version 2.0, Thermo Scientific）。蔗糖组根据建库结果设定单个样品 Data Independent Acquisition(DIA)模式的 36 个可变窗口的 DIA 方法；甜菊糖苷、安赛蜜、三氯蔗糖组根据建库结果设定单个样品 Data Independent Acquisition(DIA)模式的 39 个可变窗口的 DIA 方法。单个样品以 DIA 模式进行质谱分析，采用新建立的 DIA 方法进行 DIA 采集数据，生成 raw 文件。

2.5 Label-free DIA 定量分析

将 DIA 模式下采集的单个样品 raw 文件导入 Spectronaut Pulsar(Biognosys AG, Switzerland)软件进行分析。由 MS2 中各片段离子的峰面积相加，计算肽段丰度。由各自的肽段丰度相加计算蛋白质丰度。

2.6 数据分析

每个样本进行 3 次技术重复，取平均值进行统计学分析。

本实验进行成组分析，将食用甜味物质后和食用甜味物质前进行比较，筛选差异蛋白。差异蛋白筛选条件为：组间变化倍数（FC, Fold change）≥1.5 或 ≤0. 67，双尾非配对

t 检验分析的 P 值<0.05。筛选到的差异蛋白通过 Uniprot 网站（https://www.uniprot.org/）和 DAVID 数据库(https://david.ncifcrf.gov/)分析。在 Pubmed 数据库（https://pubmed.ncbi.nlm.nih.gov）中检索脑奖励回路相关文献，总结参与脑奖励回路的相关蛋白，在成组分析的差异蛋白中检索已被报道的脑奖励回路相关蛋白。

同时本实验进行单只小鼠食用甜味剂后和食用甜味物质前的比较分析，筛选差异蛋白，差异蛋白筛选条件为：组间变化倍数（FC, Fold change）≥ 1.5 或 ≤ 0.67，双尾非配对 t 检验分析的 P 值 < 0.05；统计 5 个样本共有的共有差异蛋白。筛选到的共有差异蛋白通过 Uniprot 网站（https://www.uniprot.org/）分析，并在单只小鼠尿液蛋白质组差异蛋白中检索已被报道的脑奖励回路相关蛋白。

3 实验结果与讨论

3.1 蔗糖组尿液蛋白质组成组分析

3.1.1 差异蛋白

将蔗糖实验组与蔗糖对照组尿液蛋白质组进行比较，筛选差异蛋白条件为：FC≥1.5 或≤0.67，双尾非配对 t 检验 P<0.05。结果表明，蔗糖实验组与蔗糖对照组相比，可以鉴定到 65 个差异蛋白，将差异蛋白按 FC 由小到大的顺序排列，通过 Uniprot 进行检索，结果如表 1 所示。

表 1 蔗糖实验组与蔗糖对照组小鼠尿液蛋白质组差异蛋白

Uniprot ID	Protein name	Fold change	Trend	P value
O09131	Glutathione S-transferase omega-1	0.231	↓	2.73E-04
P11087	Collagen alpha-1(I) chain	0.256	↓	4.09E-02
A1L317	Keratin, type I cytoskeletal 24	0.266	↓	4.50E-02
Q9R0M4	Podocalyxin	0.268	↓	4.56E-04
Q5FW60	Major urinary protein 20	0.373	↓	4.76E-02
P11680	Properdin	0.374	↓	2.10E-02
P19001	Keratin, type I cytoskeletal 19	0.379	↓	3.15E-02
P41272	CD27 antigen	0.384	↓	2.60E-02
P35459	Lymphocyte antigen 6D	0.418	↓	4.17E-02
P11591	Major urinary protein 5	0.445	↓	2.28E-02
P05533	Lymphocyte antigen 6A-2/6E-1	0.458	↓	2.80E-02
Q00898	Alpha-1-antitrypsin 1-5	0.460	↓	2.59E-02
A2BIM8	Major urinary protein 18	0.466	↓	7.34E-04
P16092	Fibroblast growth factor receptor 1	0.467	↓	2.31E-02
P23953	Carboxylesterase 1C	0.468	↓	1.16E-02
Q9R0G6	Cartilage oligomeric matrix protein	0.468	↓	3.41E-03
Q9DAK9	14 kDa phosphohistidine phosphatase	0.486	↓	1.38E-02
P06909	Complement factor H	0.497	↓	2.30E-02
P97426	Eosinophil cationic protein 1	0.523	↓	1.31E-02
P03953	Complement factor D	0.526	↓	2.98E-02
P11152	Lipoprotein lipase	0.540	↓	3.12E-02
P31786	Acyl-CoA-binding protein	0.548	↓	2.56E-02

P04939	Major urinary protein 3	0.573	↓	2.17E-02
Q3U962	Collagen alpha-2(V) chain	0.576	↓	2.62E-02
Q3UDR8	Protein YIPF3	0.578	↓	4.20E-02
Q80YX1	Tenascin	0.596	↓	2.68E-03
P57096	Prostate stem cell antigen	0.598	↓	2.81E-02
P11276	Fibronectin	0.630	↓	3.24E-02
P06797	Cathepsin L1	0.638	↓	3.41E-02
P12023	Amyloid-beta precursor protein	0.661	↓	2.07E-02
Q64729	TGF-beta receptor type-1	0.665	↓	3.09E-02
Q8K209	Adhesion G-protein coupled receptor G1	0.666	↓	1.66E-02
Q8BND5	Sulfhydryl oxidase 1	1.502	↑	3.94E-02
P13020	Gelsolin	1.630	↑	3.55E-02
P21614	Vitamin D-binding protein	1.632	↑	8.31E-03
P13634	Carbonic anhydrase 1	1.646	↑	2.30E-02
P49183	Deoxyribonuclease-1	1.680	↑	3.33E-02
P07758	Alpha-1-antitrypsin 1-1	1.742	↑	2.30E-02
P06869	Urokinase-type plasminogen activator	1.776	↑	2.30E-02
P16675	Lysosomal protective protein	1.860	↑	4.50E-02
Q99N23	Carbonic anhydrase 15	1.913	↑	2.13E-02
Q9QWR8	Alpha-N-acetylgalactosaminidase	1.920	↑	4.82E-02
P46412	Glutathione peroxidase 3	1.986	↑	6.46E-03
Q8QZR4	Out at first protein homolog	2.022	↑	3.24E-02
P99029	Peroxiredoxin-5, mitochondrial	2.063	↑	8.14E-03
P00920	Carbonic anhydrase 2	2.160	↑	3.97E-02
Q61147	Ceruloplasmin	2.178	↑	9.42E-03
Q921I1	Serotransferrin	2.240	↑	4.35E-02
P63101	14-3-3 protein zeta/delta	2.368	↑	1.72E-03
P11859	Angiotensinogen	2.424	↑	3.88E-02
Q61391	Enkephalinase	2.647	↑	3.84E-04
Q8R0I0	Angiotensin-converting enzyme 2	2.722	↑	4.76E-02
Q8VCR7	Protein ABHD14B	2.860	↑	4.53E-02
Q01768	Nucleoside diphosphate kinase B	2.914	↑	4.30E-02
E9PV24	Fibrinogen alpha chain	2.939	↑	2.20E-02
O88322	Nidogen-2	2.985	↑	1.00E-02
Q7M6Z0	Reticulon-4 receptor-like 2	3.767	↑	2.36E-02
P04940	Ig kappa chain V-VI region NQ2-17.4.1	4.272	↑	4.32E-02
P28665	Murinoglobulin-1	4.719	↑	3.44E-02
Q9DBJ1	Phosphoglycerate mutase 1	4.996	↑	5.83E-03
P10126	Elongation factor 1-alpha 1	5.152	↑	4.65E-02
P26262	Plasma kallikrein	6.801	↑	1.67E-03
P97384	Annexin A11	7.045	↑	4.12E-02
P45700	Mannosyl-oligosaccharide 1,2-alpha-mannosidase IA	7.290	↑	2.69E-03
P97798	Neogenin	从无到有	↑	4.29E-02

3.1.2 差异蛋白功能分析

将鉴定到的 65 个差异蛋白经过 DAVID 数据库进行了分子功能和生物学过程分析，结果如图 1 所示。


angiotensin- mediated drinking behavior
response to methanol
response to chromate
positive regulation of lipid metabolic process
heat generation
cellular response to lipid
negative regulation of lipid biosynthetic process
positive regulation of glucose metabolic process
negative regulation of lipid storage
regulation of cardiac conduction
energy reserve metabolic process
negative regulation of serine- type endopeptidase activity
positive regulation of gap junction assembly
negative regulation of insulin secretion involved in cellular response to glucose stimulus
locomotor rhythm
regulation of systemic arterial blood pressure by renin- angiotensin
plasminogen activation
positive regulation of CoA- transferase activity
blood coagulation, fibrin clot formation
negative regulation of gluconeogenesis
complement activation, alternative pathway
vascular smooth muscle cell differentiation
fibrinolysis
angiotensin- activated signaling pathway
mitochondrion morphogenesis
mesenchymal cell differentiation
response to lead ion
cellular response to testosterone stimulus
platelet aggregation
aerobic respiration
one- carbon metabolic process
collagen fibril organization
positive regulation of epithelial to mesenchymal transition
skin development
positive regulation of protein kinase B signaling
regulation of cell adhesion
ERK1 and ERK2 cascade
response to peptide hormone
response to cytokine
negative regulation of endopeptidase activity
cell- matrix adhesion
glucose homeostasis
response to estrogen
negative regulation of peptidase activity
blood coagulation
learning or memory
skeletal system development
positive regulation of inflammatory response
extracellular matrix organization
angiogenesis
kidney development
positive regulation of neuron projection development
positive regulation of gene expression
positive regulation of MAPK cascade
positive regulation of cell migration
response to bacterium
proteolysis
cell adhesion
regulation of gene expression
negative regulation of transcription, DNA- templated
BP
insulin- activated receptor activity
vitamin D binding
arylesterase activity
carbonate dehydratase activity
peptidase activator activity
heparan sulfate proteoglycan binding
hydro- lyase activity
acetylcholine receptor binding
proteoglycan binding
small molecule binding
endopeptidase inhibitor activity
pheromone binding
fibronectin binding
extracellular matrix structural constituent
serine- type endopeptidase inhibitor activity
protease binding
peptidase inhibitor activity
heparin binding
collagen binding
endopeptidase activity
odorant binding
receptor binding
peptidase activity
hydrolase activity
identical protein binding
MF
0
50
100
150
200
- log10(pvalue)
9
6
3
count
4
8
12
16

图 1 蔗糖组小鼠差异蛋白分子功能和生物学过程分析

按 p 值由小到大排序，其中排名前 30%的 18 个生物学过程中，有 12 个生物学过程显示的是糖、脂代谢和能量生产带来的变化，如：正向调节脂质代谢过程、热的产生、细胞对脂质的反应、脂质生物合成过程的负调控、葡萄糖代谢过程的正向调节、负性调节脂质储存、能量储备代谢过程、胰岛素分泌的负调控参与细胞对葡萄糖刺激的反应、糖异生的负调控、线粒体形态发生、有氧呼吸、葡萄糖稳态等；按 p 值由小到大排序，其中排名第一的分子功能是胰岛素受体激活，其余分子功能也主要与分子结合、各类酶的激活、气味结合等。这很可能与食用蔗糖导致的代谢反应相关。但是值得注意的是，差异蛋白同样富集到了神经元投射发育的正调控、学习或记忆等神经相关的生物学过程，这可能与甜味觉的喜好反应相关。

3.1.3 差异蛋白与脑奖励回路

为了进一步探究尿液蛋白质组差异蛋白是否有脑奖励回路相关的蛋白，通过 Uniprot 检索蔗糖组差异蛋白的功能和涉及的生物学过程，寻找是否与已报道的脑奖励回路中的关键蛋白相关[19]，结果如表 2 所示。

表 2 蔗糖组小鼠尿液蛋白质组差异蛋白与脑奖励回路

The Brain's Reward Circuitry	
Glutamate	√
Dopamine	
Gamma-aminobutyric acid / GABA	√
Substance P	√
Enkephalin	√

小鼠尿液蛋白质组差异蛋白中检索到有和脑奖励回路相关的蛋白。

Acyl-CoA-binding protein 能够取代神经官能症药物地西泮位于γ-氨基丁酸 A 型受体上的苯二氮卓的识别位点，能作为神经肽来调节γ-氨基丁酸受体的作用；参与到学习记忆、突触传递的正调控、神经胶质细胞增殖等生物学过程中。

Procathepsin L 在神经内分泌嗜染色质细胞分泌囊泡中，催化激素原脑啡肽加工成活性脑啡肽神经递质；参与到脑啡肽的处理、神经发育等生物学过程中。

Amyloid-beta precursor protein 作为细胞表面受体，在神经元表面执行与神经突生长、神经元粘附和轴突发生有关的生理功能；参与学习和记忆、谷氨酸受体信号通路、联想学习、神经元分化等生物学过程中。

Carbonic anhydrase 2 参与到γ-氨基丁酸能突触传递的正调节等生物学过程中。

Angiotensinogen 参与到联想学习、神经元凋亡过程的负调控等生物学过程中。

14-3-3 protein zeta/delta 在谷氨酸能突触等区域表达，参与到突触成熟的调控、突触目标的识别等生物学过程中。

Enkephalinase 具有裂解阿片肽，如：Met-enkephalin 和 Leu-enkephalin 的能力，还能催化缓激肽、Substance P 和神经紧张肽的裂解；参与到学习记忆、Substance P 分解代谢、神经发育的正调控等生物学过程中。

3.2 蔗糖组个体食用蔗糖后前尿液蛋白质组单个分析发现共有一个脑奖励回路相关蛋白

分别将 4 只小鼠食用蔗糖后的尿液蛋白质组与食用蔗糖前的尿液蛋白质组进行比较，筛选差异蛋白条件为：FC≥1.5 或≤0.67，双尾非配对 t 检验 $P<0.05$，统计 4 只小鼠共有的差异蛋白，结果如图 2 所示。

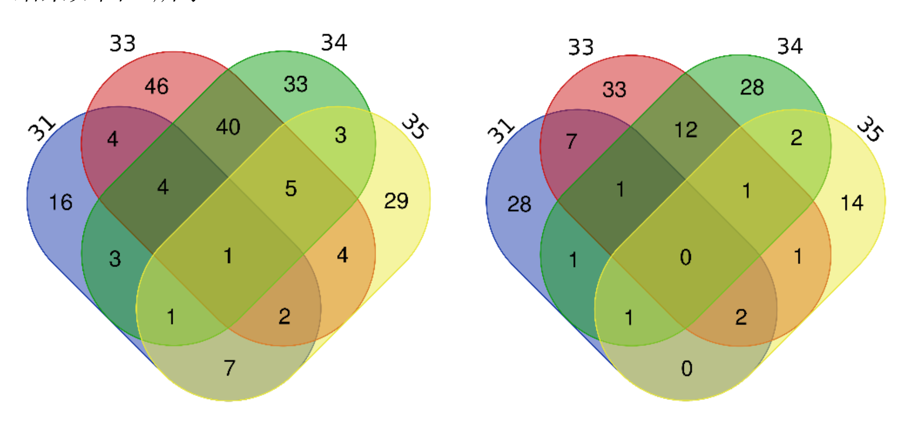


图 2 蔗糖组个体食用蔗糖后前共有上调差异蛋白（左）下调差异蛋白（右）

4 只小鼠共有 1 个上调差异蛋白。通过 Uniprot 检索蛋白的功能和涉及的生物学过程，Enkephalinase 与脑奖励回路相关，具有裂解阿片肽，如：Met-enkephalin 和 Leu-enkephalin 的能力，还能催化缓激肽、Substance P 和神经紧张肽的裂解；参与到学习记忆、Substance P 分解代谢、神经发育的正调控等生物学过程中。

同时对单个小鼠食用蔗糖后前的尿液蛋白质组差异蛋白进行检索，查找单个小鼠尿液蛋白质组差异蛋白中具有的脑奖励回路相关蛋白，如表 3 所示。

表 3 蔗糖组个体食用蔗糖后前尿液蛋白质组差异蛋白中与脑奖励回路相关的蛋白

	Glutamate	Dopamine	Gamma-aminobutyric acid GABA	Substance P	Enkephalin	Other neural correlations
31	Epidermal growth factor receptor Glutathione hydrolase 1 proenzyme Aspartate aminotransferase, cytoplasmic Heat shock cognate 71 kDa protein			Enkephalinase	Enkephalinase	Lysosomal alpha-mannosidase Ganglioside GM2 activator
33	Profilin-1 Aspartate aminotransferase, cytoplasmic Heat shock cognate 71 kDa protein Calbindin Glutathione hydrolase 1 proenzyme Actin, alpha cardiac muscle 1 Ephrin type-B receptor 2 14-3-3 protein zeta/delta Syntenin-1 Amyloid-beta precursor protein Calumenin Progranulin		Cathepsin D Carbonic anhydrase 2 Calbindin Acyl-CoA-binding protein Cadherin-13	Enkephalinase	Enkephalinase	Lysosomal alpha-mannosidase Angiotensinogen Voltage-dependent anion-selective channel protein 1
34	Profilin-1 Aspartate aminotransferase, cytoplasmic Heat shock cognate 71 kDa protein Glypican-4 Fractalkine Progranulin Tumor necrosis factor receptor superfamily member 16		Carbonic anhydrase 2 Cadherin-13	Enkephalinase	Enkephalinase	Lysosomal alpha-mannosidase
35	Calbindin Plasminogen Aspartate aminotransferase, mitochondrial		Calbindin	Enkephalinase	Enkephalinase	Angiotensinogen

3.3 甜菊糖苷组尿液蛋白质组成组分析

3.3.1 差异蛋白

将甜菊糖苷实验组与甜菊糖苷对照组尿液蛋白质组进行比较，筛选差异蛋白条件为：FC≥1.5 或≤0.67，双尾非配对 t 检验 $P<0.05$。结果表明，甜菊糖苷实验组与甜菊糖苷对照组相比，可以鉴定到 66 个差异蛋白，将差异蛋白按 FC 由小到大的顺序排列，通过 Uniprot 进行检索，结果如表 4 所示。

表 4 甜菊糖苷实验组与甜菊糖苷对照组小鼠尿液蛋白质组差异蛋白

Uniprot ID	Protein name	Fold change	Trend	P value
P15949	Kallikrein 1-related peptidase b9	0.000	↓	6.21E-03
P07141	Macrophage colony-stimulating factor 1	0.000	↓	3.44E-02
P61458	Pterin-4-alpha-carbinolamine dehydratase	0.000	↓	3.93E-02
O08709	Peroxiredoxin-6	0.023	↓	3.25E-02
Q8C110	SLIT and NTRK-like protein 6	0.060	↓	7.73E-03
Q8BX35	Tumor necrosis factor receptor superfamily member 27	0.091	↓	2.57E-02
Q64105	Sepiapterin reductase	0.103	↓	2.85E-02
O35215	D-dopachrome decarboxylase	0.111	↓	4.87E-03
Q9DAS9	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-12	0.122	↓	4.52E-02
P14152	Malate dehydrogenase, cytoplasmic	0.190	↓	3.07E-03
P52760	2-iminobutanoate/2-iminopropanoate deaminase	0.194	↓	2.08E-02
P70441	Na(+)/H(+) exchange regulatory cofactor NHE-RF1	0.197	↓	3.06E-02
Q9ES90	G-protein coupled receptor 35	0.216	↓	6.32E-03
Q7TSK7	ADAMTS-like protein 2	0.226	↓	9.15E-03
P02301	Histone H3.3C	0.241	↓	3.48E-02
Q3V0K9	Plastin-1	0.257	↓	4.16E-02
Q9Z2J0	Solute carrier family 23 member 1	0.260	↓	3.79E-02
Q3UMW8	Ceroid-lipofuscinosis neuronal protein 5 homolog	0.274	↓	4.39E-02
Q99PT1	Rho GDP-dissociation inhibitor 1	0.279	↓	4.43E-02
Q61088	Frizzled-4	0.297	↓	3.42E-02
Q01721	Growth arrest-specific protein 1	0.321	↓	9.62E-03
P35505	Fumarylacetoacetase	0.370	↓	2.35E-02
Q9WVJ9	EGF-containing fibulin-like extracellular matrix protein 2	0.373	↓	1.35E-02
P62806	Histone H4	0.378	↓	9.67E-03
P51655	Glypican-4	0.387	↓	2.87E-02
Q60928	Glutathione hydrolase 1 proenzyme	0.429	↓	1.05E-02
Q91WR6	Glycoprotein integral membrane protein 1	0.442	↓	4.98E-02
Q08761	Vitamin K-dependent protein S	0.450	↓	4.46E-02
P48441	Alpha-L-iduronidase	0.457	↓	2.25E-02
P10649	Glutathione S-transferase Mu 1	0.513	↓	4.86E-03
Q921W8	Secreted and transmembrane protein 1A	0.571	↓	2.82E-02
P99029	Peroxiredoxin-5, mitochondrial	0.573	↓	1.56E-02
P62737	Actin, aortic smooth muscle	0.644	↓	4.24E-02
P35441	Thrombospondin-1	1.542	↑	2.51E-02
P55292	Desmocollin-2	1.542	↑	3.35E-02
P18181	CD48 antigen	1.570	↑	2.65E-02
Q64726	Zinc-alpha-2-glycoprotein	1.657	↑	2.93E-02
Q9R1E6	Ectonucleotide pyrophosphatase/phosphodiesterase family member 2	1.678	↑	1.37E-02
Q61147	Ceruloplasmin	1.685	↑	4.99E-02
Q61398	Procollagen C-endopeptidase enhancer 1	1.690	↑	2.47E-04

Q03404	Trefoil factor 2	1.702	↑	9.86E-04
P97792	Coxsackievirus and adenovirus receptor homolog	1.782	↑	4.22E-02
Q80YX1	Tenascin	1.806	↑	2.54E-02
P11438	Lysosome-associated membrane glycoprotein 1	1.809	↑	1.96E-02
P13597	Intercellular adhesion molecule 1	1.812	↑	7.73E-04
P03953	Complement factor D	1.927	↑	5.83E-03
B1AZI6	THO complex subunit 2	1.991	↑	4.97E-02
P19221	Prothrombin	1.996	↑	7.78E-03
P07361	Alpha-1-acid glycoprotein 2	2.005	↑	3.76E-02
Q3UVY5	Pecanex-like protein 4	2.017	↑	2.27E-02
Q8VCS0	N-acetylmuramoyl-L-alanine amidase	2.118	↑	1.36E-02
P51910	Apolipoprotein D	2.203	↑	1.96E-02
P00687	Alpha-amylase 1	2.225	↑	4.19E-02
P57096	Prostate stem cell antigen	2.261	↑	2.88E-02
Q8BWP8	Beta-1,4-glucuronyltransferase 1	2.444	↑	4.38E-02
Q9Z0M9	Interleukin-18-binding protein	2.570	↑	3.32E-03
Q8K1H9	Odorant-binding protein 2a	2.574	↑	2.48E-02
Q9DAU7	WAP four-disulfide core domain protein 2	2.575	↑	4.22E-03
Q9JKR6	Hypoxia up-regulated protein 1	2.702	↑	2.14E-02
Q9D7R7	Gastricsin	3.323	↑	1.62E-02
Q9JI02	Secretoglobin family 2B member 20	4.188	↑	2.74E-02
O54965	E3 ubiquitin-protein ligase RNF13	4.648	↑	7.05E-03
Q60823	RAC-beta serine/threonine-protein kinase	5.194	↑	4.73E-02
Q6PGG6	Guanine nucleotide-binding protein-like 3-like protein	5.833	↑	6.43E-03
Q8R0W0	Epiplakin	9.865	↑	4.89E-02
Q9Z138	Tyrosine-protein kinase transmembrane receptor ROR2	13.671	↑	3.22E-02

3.3.2 差异蛋白功能分析

将鉴定到的 66 个差异蛋白经过 DAVID 数据库进行分子功能和生物学过程分析，结果如图 3 所示。

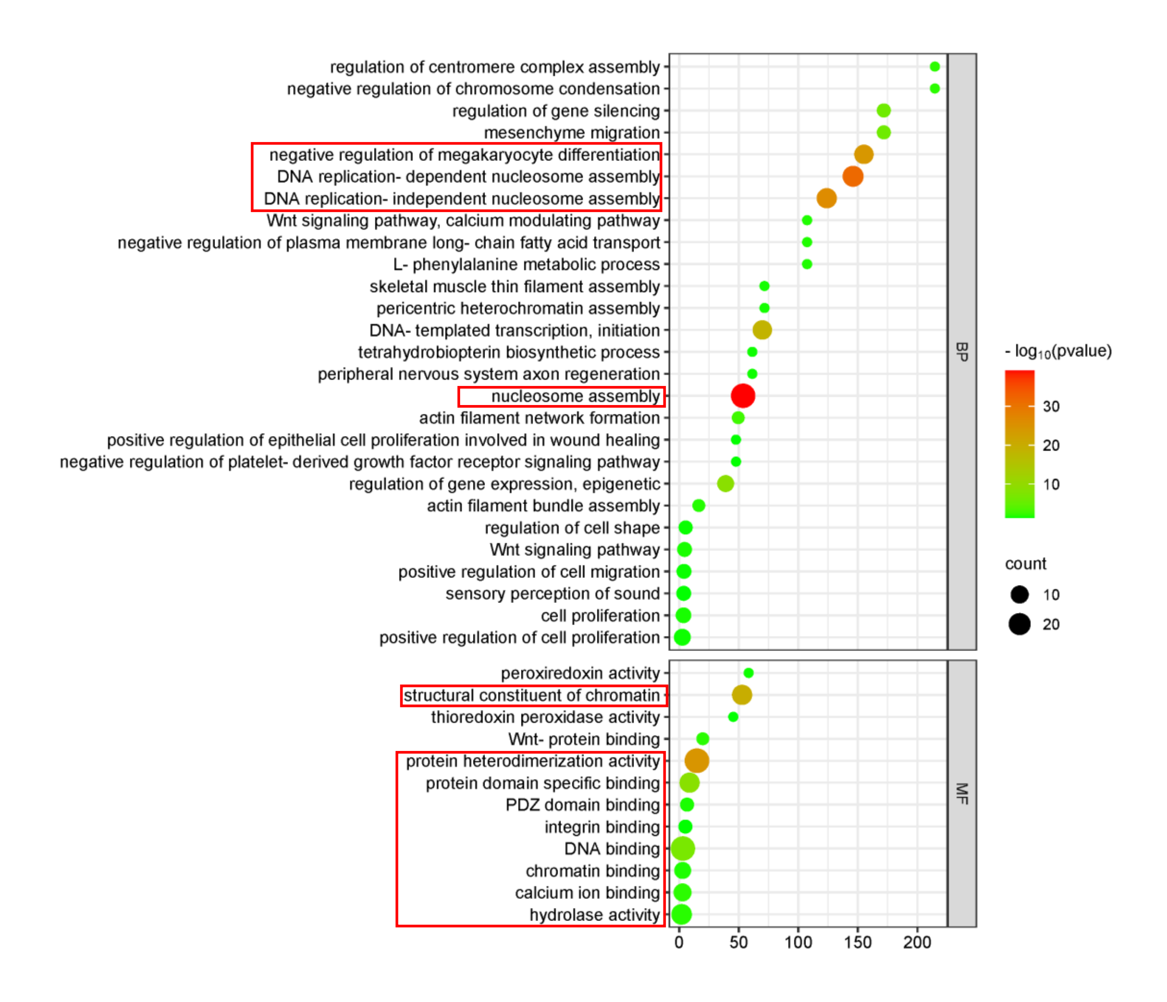


图 3 甜菊糖苷组小鼠差异蛋白分子功能和生物学过程分析

按 p 值由小到大排序，其中排名前 50%的 13 个生物学过程中，有 11 个生物学过程显示的核小体组装、基因表达、细胞分裂带来的变化，如：核小体组装、DNA 复制依赖的核小体组装、DNA 复制非依赖的核小体组装、DNA 模板转录，起始、基因表达调控，表观遗传学、基因沉默的调控、肌动蛋白丝网形成、染色体凝聚负调控、着丝粒复合体组装的调控、肌动蛋白丝束组装等，与蔗糖组展示出的大量代谢相关的生物学过程不同，这是首次看到小鼠自主食用甜菊糖苷后，尿液蛋白质组差异蛋白中出现核小体相关的变化，且其他甜味剂中没有出现这样的变化；按 p 值由小到大排序，其中排名前四的分子功能是蛋白质异源二聚化活性、染色质的结构成分、蛋白质结构域特异性结合、DNA 结合，分子功能也主要与 DNA 和蛋白质的活动相关，蔗糖组显著出现的胰岛素相关分子功能在甜菊糖苷组未显示。但是值得注意的是，差异蛋白同样富集到了外周神经系统轴突再生这种神经相关的生物学过程，这可能与甜味觉的喜好反应相关。

3.3.3 差异蛋白与脑奖励回路

为了进一步探究尿液蛋白质组差异蛋白是否具脑奖励回路相关的蛋白，通过 Uniprot 检索甜菊糖苷组差异蛋白的功能和涉及的生物学过程，寻找是否与已报道的脑奖励回路中的关键蛋白相关，结果如表 5 所示。

表 5 甜菊糖苷组小鼠尿液蛋白质组差异蛋白与脑奖励回路

The Brain’s Reward Circuitry	
Glutamate	√
Dopamine	√
Gamma-aminobutyric acid / GABA	√
Substance P	
Enkephalin	

小鼠尿液蛋白质组差异蛋白中检索到有和脑奖励回路相关的蛋白。

Sepiapterin reductase 参与到神经元分化过程中的细胞形态发生、多巴胺代谢等生物学过程中。

Na(+)/H(+) exchange regulatory cofactor NHE-RF1 具有多巴胺受体结合、γ-氨基丁酸跨膜转运等功能，参与到腺苷酸环化酶激活多巴胺受体信号通路、磷脂酶 c 激活多巴胺受体信号通路等生物学过程中。

Frizzled-4 在谷氨酸能突触中表达，参与到树突形态发生的正调控、神经元投射树化的正调控等生物学过程中。

Glypican-4 在谷氨酸能突触、突触前膜等位置表达，参与到神经递质受体定位对突触后特化膜的调节、突触前组装调控、突触膜黏附等生物学过程中。

Glutathione hydrolase 1 proenzyme 具有谷胱甘肽水解酶活性，参与谷胱甘肽的分解代谢、谷氨酸的代谢过程和对酒精的反应。

Tyrosine-protein kinase transmembrane receptor ROR2 在树突、谷氨酸能突触、神经元胞体等位置中表达，参与到星形胶质细胞发育、化学突触传递的调节、谷氨酸能突触传递的正调控、神经元投射发育的正调控、突触后组织的调控等生物学过程中。

3.4 甜菊糖苷组个体食用甜菊糖苷后前尿液蛋白质组单个分析

分别将 5 只小鼠食用甜菊糖苷后的尿液蛋白质组与食用甜菊糖苷前的尿液蛋白质组进行比较，筛选差异蛋白条件为：FC≥1.5 或≤0.67，双尾非配对 t 检验 $P<0.05$，统计 5 只小鼠共有的差异蛋白，结果如图 4 所示。

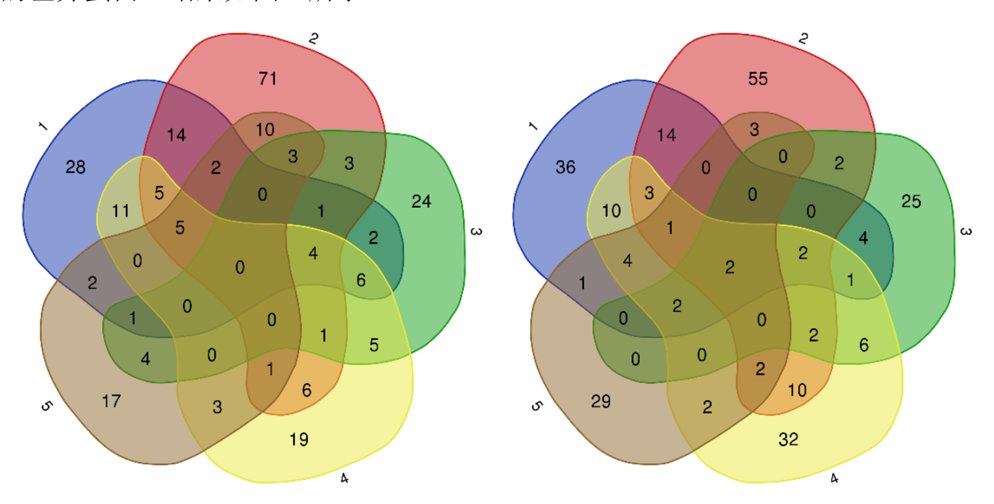


图 4 甜菊糖苷组个体食用甜菊糖苷后前共有上调差异蛋白（左）下调差异蛋白（右）

5 只小鼠共有 2 个下调差异蛋白，分别为 2-iminobutanoate/2-iminopropanoate deaminase 和 Glutathione hydrolase 1 proenzyme。通过 Uniprot 检索蛋白的功能和涉及的生物学过程，

2-iminobutanoate/2-iminopropanoate deaminase 具有催化正常代谢过程中形成的烯胺/亚胺中间体的水解脱胺、促进核糖核酸酶 P/MRP 复合体的募集来促进核糖核酸内裂解的功能，参与到 mRNA 分解、翻译负调控等生物学过程中；Glutathione hydrolase 1 proenzyme 具有谷胱甘肽水解酶活性，参与谷胱甘肽的分解代谢、谷氨酸的代谢过程和对酒精的反应。

同时对单个小鼠食用甜菊糖苷后前的尿液蛋白质组差异蛋白进行检索，查找单个小鼠尿液蛋白质组差异蛋白中具有的脑奖励回路相关蛋白，如表 6 所示。

表 6 甜菊糖苷组个体食用甜菊糖苷后前尿液蛋白质组差异蛋白中与脑奖励回路相关的蛋白

	Glutamate	Dopamine	Gamma-aminobutyric acid GABA	Substance P	Enkephalin	Other neural correlations
1	Progranulin Interleukin-1 receptor accessory protein Cadherin-1 Neuroplastin Glutathione hydrolase 1 proenzyme Glyceraldehyde-3-phosphate dehydrogenase Calmodulin-1 Dystroglycan 1 Amino acid transporter heavy chain SLC3A2 Amino acid transporter heavy chain SLC3A1 Calbindin	Stromal cell-derived factor 1	Neuroplastin Dystroglycan 1 Calbindin		Procathepsin L	ATP-binding cassette sub-family A member 7 Voltage-dependent anion-selective channel protein 1 Alpha-L-iduronidase Angiotensinogen Ganglioside GM2 activator
2	Cadherin-11 Interleukin-1 receptor accessory protein Fractalkine SPARC-like protein 1 Cadherin-2 Gamma-glutamyl hydrolase Glyceraldehyde-3-phosphate dehydrogenase Heat shock cognate 71 kDa protein Glutathione hydrolase 1 proenzyme Frizzled-4 Glypican-4 Dystroglycan 1 Amino acid transporter heavy chain SLC3A2 Myc box-dependent-interacting protein 1 Sodium/potassium-transporting ATPase subunit alpha-3 Aggrecan core protein	Sodium/potassium-transporting ATPase subunit alpha-3 Sepiapterin reductase	Carbonic anhydrase 2 Dystroglycan 1 Aggrecan core protein			Ganglioside GM2 activator Alpha-L-iduronidase
3	Progranulin Cadherin-11 Fractalkine Glutathione hydrolase 1 proenzyme Glyceraldehyde-3-phosphate dehydrogenase Frizzled-4 N-alpha-acetyltransferase 10	Cadherin EGF LAG seven-pass G-type receptor 3 Aldehyde dehydrogenase, mitochondrial	Carbonic anhydrase 2			Voltage-dependent anion-selective channel protein 1 Angiotensinogen
4	Progranulin Cadherin-1 Ephrin type-B receptor 2 Glutathione hydrolase 1 proenzyme Glyceraldehyde-3-phosphate dehydrogenase Calmodulin-1 Frizzled-4 Glypican-4 Ornithine aminotransferase, mitochondrial Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial (HCDH)	Stromal cell-derived factor 1				Voltage-dependent anion-selective channel protein 1 Angiotensinogen
5	Cadherin-11 Glutathione hydrolase 1 proenzyme Calmodulin-1 Glypican-4 Progranulin		Carbonic anhydrase 2			Angiotensinogen ATP-binding cassette sub-family A member 7 Voltage-dependent anion-selective channel protein 1

3.5 安赛蜜组尿液蛋白质组成组分析

3.5.1 安赛蜜组总尿液蛋白质组非监督聚类分析

对安赛蜜实验组和安赛蜜对照组的总尿液蛋白质组进行非监督聚类分析，结果如图 5 所示。通过总蛋白质组即可对小鼠食用安赛蜜的前后进行区分，食用安赛蜜后小鼠尿液蛋白质组发生了显著变化。

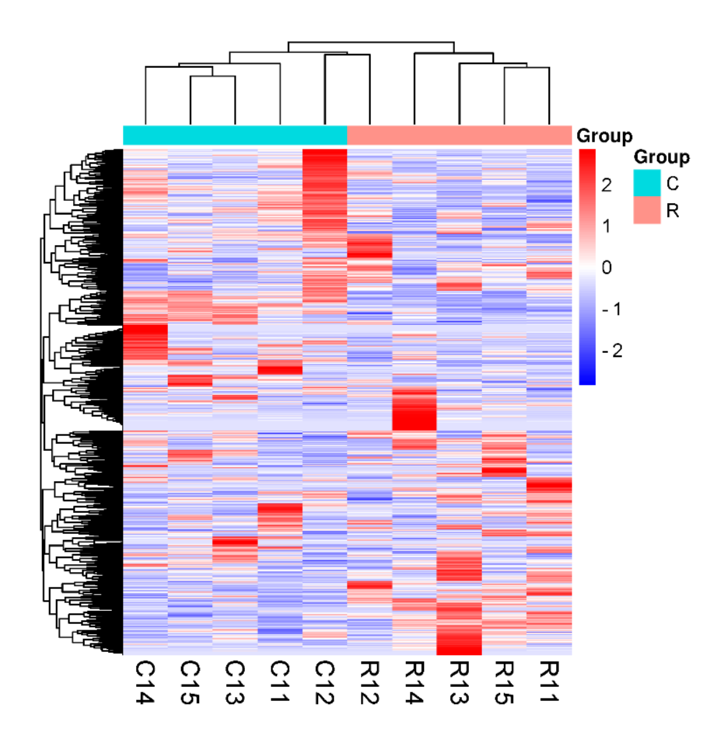


图 5 安赛蜜组总尿液蛋白质组非监督聚类

3.5.2 差异蛋白

安赛蜜实验组与安赛蜜对照组尿液蛋白质进行比较，筛选差异蛋白条件为：FC≥1.5 或≤0.67，双尾非配对 t 检验 P<0.05。结果表明，安赛蜜实验组与安赛蜜对照组相比，可以鉴定到 93 个差异蛋白，将差异蛋白按 FC 由小到大的顺序排列，通过 Uniprot 进行检索，结果如表 7 所示。

表 7 安赛蜜实验组与安赛蜜对照组小鼠尿液蛋白质组差异蛋白

Uniprot ID	Protein name	Fold change	Trend	P value
O35215	D-dopachrome decarboxylase	0.00	↓	7.84E-04
Q61247	Alpha-2-antiplasmin	0.00	↓	1.58E-02
Q14BI7	ATP-dependent RNA helicase TDRD9	0.00	↓	2.31E-02
P55849	Desmocollin-1	0.00	↓	2.50E-02
Q8VEM8	Phosphate carrier protein, mitochondrial	0.00	↓	2.64E-02
P35802	Neuronal membrane glycoprotein M6-a	0.00	↓	4.52E-02
P52760	2-iminobutanoate/2-iminopropanoate deaminase	0.01	↓	1.50E-02
P61458	Pterin-4-alpha-carbinolamine dehydratase	0.02	↓	2.37E-06
Q9DAS9	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-12	0.03	↓	9.92E-03
O09051	Guanylate cyclase activator 2B	0.08	↓	2.79E-02
P99029	Peroxiredoxin-5, mitochondrial	0.09	↓	1.17E-03
P70441	Na(+)/H(+) exchange regulatory cofactor NHE-RF1	0.10	↓	1.74E-02
P35505	Fumarylacetoacetase	0.12	↓	3.36E-03

Q9JLT2	Trehalase	0.15	↓	2.72E-02
P26043	Radixin	0.19	↓	2.57E-02
Q7M6Z0	Reticulon-4 receptor-like 2	0.22	↓	2.35E-02
P12658	Calbindin	0.22	↓	4.78E-02
Q8K426	Resistin-like gamma	0.25	↓	1.35E-02
Q60928	Glutathione hydrolase 1 proenzyme	0.25	↓	1.24E-02
P81117	Nucleobindin-2	0.26	↓	1.18E-02
Q08761	Vitamin K-dependent protein S	0.30	↓	5.15E-03
Q91XE4	N-acyl-aromatic-L-amino acid amidohydrolase (carboxylate-forming)	0.30	↓	1.43E-02
Q9ES90	G-protein coupled receptor 35	0.32	↓	7.29E-03
Q64449	C-type mannose receptor 2	0.38	↓	8.50E-03
P10649	Glutathione S-transferase Mu 1	0.39	↓	3.48E-02
Q9QXD6	Fructose-1,6-bisphosphatase 1	0.39	↓	4.16E-02
O09133	Submaxillary gland androgen-regulated protein 2, isoform alpha	0.42	↓	3.62E-02
Q60932	Voltage-dependent anion-selective channel protein 1	0.43	↓	1.69E-03
Q6RKD8	Leucine-rich repeat transmembrane protein FLRT1	0.45	↓	3.82E-03
Q91WV7	Neutral and basic amino acid transport protein rBAT	0.49	↓	3.12E-02
O08992	Syntenin-1	0.49	↓	3.30E-03
Q9CQ60	6-phosphogluconolactonase	0.50	↓	3.36E-02
Q8VC60	Beta-galactosidase-1-like protein	0.50	↓	3.82E-03
Q9JHY4	WAP four-disulfide core domain protein 15B	0.51	↓	7.56E-04
Q91ZI0	Cadherin EGF LAG seven-pass G-type receptor 3	0.52	↓	5.31E-04
P70699	Lysosomal alpha-glucosidase	0.54	↓	6.08E-05
O89051	Integral membrane protein 2B	0.54	↓	2.11E-02
Q02819	Nucleobindin-1	0.55	↓	5.13E-03
P48441	Alpha-L-iduronidase	0.55	↓	4.30E-02
Q9CZT5	Vasorin	0.55	↓	3.47E-02
P11152	Lipoprotein lipase	0.57	↓	9.54E-03
Q8C050	Ribosomal protein S6 kinase alpha-5	0.57	↓	2.18E-02
Q9QWR8	Alpha-N-acetylgalactosaminidase	0.58	↓	1.14E-03
O35298	Acyloxyacyl hydrolase	0.60	↓	7.23E-03
P17563	Methanethiol oxidase	0.63	↓	1.31E-02
P23780	Beta-galactosidase	0.63	↓	1.48E-03
O35448	Lysosomal thioesterase PPT2	0.63	↓	1.34E-03
Q91Y97	Fructose-bisphosphate aldolase B	0.65	↓	1.80E-02
Q9DC11	Plexin domain-containing protein 2	1.54	↑	4.80E-02
Q91V08	C-type lectin domain family 2 member D	1.58	↑	1.85E-02

P01027	Complement C3	1.60	↑	2.12E-03
Q61398	Procollagen C-endopeptidase enhancer 1	1.61	↑	3.20E-03
Q9R1E6	Ectonucleotide pyrophosphatase/phosphodiesterase family member 2	1.67	↑	1.91E-02
Q80YX1	Tenascin	1.68	↑	4.06E-02
Q61147	Ceruloplasmin	1.72	↑	3.04E-03
P14094	Sodium/potassium-transporting ATPase subunit beta-1	1.72	↑	3.17E-03
O35305	Tumor necrosis factor receptor superfamily member 11A	1.84	↑	4.66E-02
P28665	Murinoglobulin-1	1.89	↑	1.76E-02
Q9ESD1	Prostasin	1.89	↑	1.78E-02
P54763	Ephrin type-B receptor 2	1.92	↑	4.38E-02
Q3UVY5	Pecanex-like protein 4	1.95	↑	2.08E-02
P18572	Basigin	1.96	↑	4.27E-02
P31809	Carcinoembryonic antigen-related cell adhesion molecule 1	1.99	↑	1.17E-02
P51910	Apolipoprotein D	2.01	↑	3.56E-03
P20029	Endoplasmic reticulum chaperone BiP	2.04	↑	3.30E-02
P20108	Thioredoxin-dependent peroxide reductase, mitochondrial	2.06	↑	3.94E-02
P13597	Intercellular adhesion molecule 1	2.08	↑	7.88E-04
Q05909	Receptor-type tyrosine-protein phosphatase gamma	2.11	↑	4.39E-03
P61110	Kidney androgen-regulated protein	2.14	↑	1.85E-02
Q9CQW3	Vitamin K-dependent protein Z	2.21	↑	6.07E-03
P35846	Folate receptor alpha	2.22	↑	4.92E-02
P08249	Malate dehydrogenase, mitochondrial	2.24	↑	1.01E-02
Q9D8B7	Junctional adhesion molecule C	2.28	↑	2.81E-02
P48614	Protein Wnt-10b	2.29	↑	2.92E-02
P57096	Prostate stem cell antigen	2.69	↑	9.27E-03
Q00724	Retinol-binding protein 4	2.85	↑	3.94E-02
Q61495	Desmoglein-1-alpha	2.94	↑	2.13E-02
Q08423	Trefoil factor 1	3.15	↑	3.49E-02
P26618	Platelet-derived growth factor receptor alpha	3.32	↑	4.83E-02
P35461	Lymphocyte antigen 6G (Fragment)	3.75	↑	2.64E-02
P04441	H-2 class II histocompatibility antigen gamma chain	4.28	↑	4.45E-02
Q9R007	C-type lectin domain family 5 member A	4.39	↑	6.03E-06
Q64729	TGF-beta receptor type-1	4.55	↑	4.63E-02
Q9DAU7	WAP four-disulfide core domain protein 2	4.59	↑	1.78E-02
Q811B3	A disintegrin and metalloproteinase with	4.61	↑	3.82E-02

	thrombospondin motifs 12			
P16092	Fibroblast growth factor receptor 1	5.44	↑	4.02E-02
P31725	Protein S100-A9	5.53	↑	1.83E-02
Q60823	RAC-beta serine/threonine-protein kinase	5.83	↑	3.06E-02
Q9CR33	MANSC domain-containing protein 1	6.10	↑	1.68E-02
O54965	E3 ubiquitin-protein ligase RNF13	10.79	↑	4.26E-02
Q9JM99	Proteoglycan 4	16.79	↑	3.09E-02
P51885	Lumican	25.22	↑	3.02E-03
P07901	Heat shock protein HSP 90-alpha	30.83	↑	3.49E-02

3.5.3 差异蛋白功能分析

将鉴定到的 93 个差异蛋白经过 DAVID 数据库进行了分子功能和生物学过程分析，结果如图 6 所示。



图 6 安赛蜜组小鼠差异蛋白分子功能和生物学过程分析

按 p 值由小到大排序，其中排名前 30%的 10 个生物学过程中，有 3 个生物学过程涉及到了糖、脂代谢，如：碳水化合物代谢过程、脂质代谢过程、代谢过程等，比例要小于蔗糖组；按 p 值由小到大排序，其中排名第三的分子功能与水解酶活性相关，尤其是糖基键水解酶，蔗糖组显著出现的胰岛素相关分子功能在安赛蜜组则未显示。值得注意的是，差异蛋白同样富集到了外周神经系统轴突再生等神经相关的生物学过程，这可能与甜味觉的喜好反应相关。

3.5.4 差异蛋白与脑奖励回路

为了进一步探究尿液蛋白质组差异蛋白是否有脑奖励回路相关的蛋白，通过 Uniprot 检索安赛蜜组差异蛋白的功能和涉及的生物学过程，寻找是否与已报道的脑奖励回路中的关键蛋白相关，结果如表 8 所示。

表 8 安赛蜜组小鼠尿液蛋白质组差异蛋白与脑奖励回路

The Brain’s Reward Circuitry	
Glutamate	√
Dopamine	√
Gamma-aminobutyric acid / GABA	√
Substance P	
enkephalin	

小鼠尿液蛋白质组差异蛋白中检索到有和脑奖励回路相关的蛋白。

Neuronal membrane glycoprotein M6-a 在神经元可塑性中起作用，参与神经分化，包括神经干细胞的分化和迁移，参与神经突和丝状足的生长，丝状足的运动，可能还参与突触的形成。在轴突生长锥、树突棘、谷氨酸能突触、神经元胞体、平行纤维与浦肯野细胞突触、突触前活性区膜等区域表达，参与到神经元迁移、神经元投射发育、神经元投射形态发生、突触组织的调控、突触组装等生物学过程中。

Na(+)/H(+) exchange regulatory cofactor NHE-RF1 具有多巴胺受体结合、γ-氨基丁酸跨膜转运等功能，参与到腺苷酸环化酶激活多巴胺受体信号通路、磷脂酶 c 激活多巴胺受体信号通路等生物学过程中。

Calbindin 在轴突、树突、树突棘、gamma-aminobutyric acid 能突触、谷氨酸能突触、神经元胞体等区域中表达，参与到突触可塑性调控、通过钙离子结合调节突触前后胞质钙离子浓度、突触前胞质钙离子浓度的调节、长期记忆、短期记忆等生物学过程中。

Glutathione hydrolase 1 proenzyme 具有谷胱甘肽水解酶活性，参与谷胱甘肽的分解代谢、谷氨酸的代谢过程和对酒精的反应。

Amino acid transporter heavy chain SLC3A1 参与到谷氨酸的跨膜转运。

Syntenin-1 参与到嗜离子性谷氨酸受体结合和突触前组装等生物学过程中。

Cadherin EGF LAG seven-pass G-type receptor 3 是神经系统形成过程中，在细胞间信号传导中起重要作用的受体；参与到轴突束颤、多巴胺能神经元轴突引导、运动神经元迁移、平面细胞极性通路参与轴突引导、5-羟色胺能神经元轴突引导等生物学过程中。

Ephrin type-B receptor 2 在轴突、树突、树突棘、谷氨酸能突触、神经元胞体、突触后膜、突触前膜、突触等区域表达，具有调节树突棘发育成熟，刺激兴奋性突触形成的功能。参与到轴突发生、中枢神经系统投射神经元轴突发生、联合神经元轴突引导、树突棘形态发生、学习和记忆、神经元投射收缩、树突棘形态发生的正调控、长期神经元突触可塑性的正调节、长期突触增强的正调节、突触组装的正调控、突触可塑性的正向调节、突触后膜组装、轴突发生调控、神经突触可塑性调控、突触组装的调控、跨突触复合体对突触传递的调节作用等生物学过程中。

3.6 安赛蜜组个体食用安赛蜜后前尿液蛋白质组单个分析

分别将 5 只小鼠食用安赛蜜后的尿液蛋白质组与食用安赛蜜前的尿液蛋白质组进行比较，筛选差异蛋白条件为：FC≥1.5 或≤0.67，双尾非配对 t 检验 P<0.05，统计 5 只小鼠共有

的差异蛋白，结果如图 7 所示。

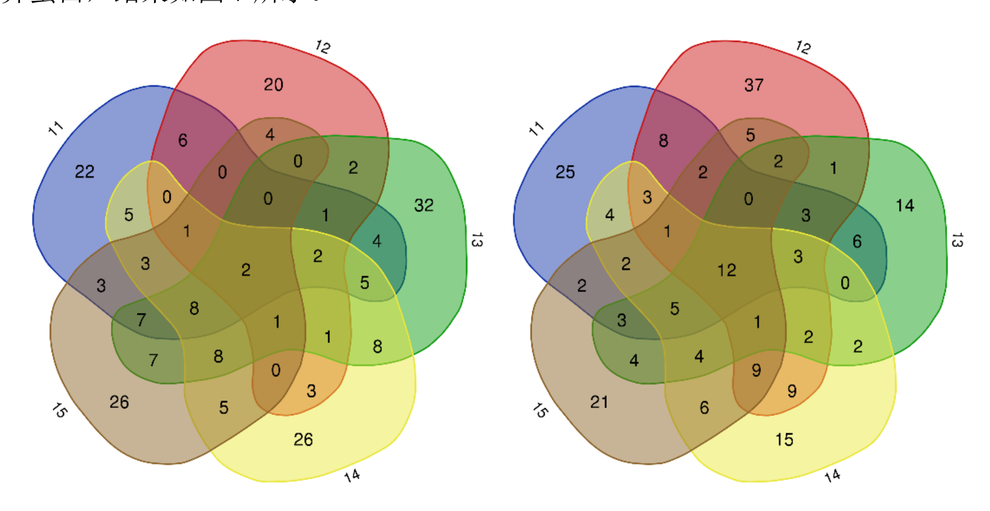


图 7 安赛蜜组个体食用安赛蜜后前共有上调差异蛋白（左）下调差异蛋白（右）

5 只小鼠共有 2 个上调差异蛋白，12 个下调差异蛋白，如表 7 所示。

表 7 安赛蜜组个体食用安赛蜜后前共有差异蛋白

Uniprot ID	Protein name	Trend
Q06318	Uteroglobin	↓
Q91XE4	N-acyl-aromatic-L-amino acid amidohydrolase	↓
P70699	Lysosomal alpha-glucosidase	↓
Q9JHY4	WAP four-disulfide core domain protein 15B	↓
P70441	Na(+)/H(+) exchange regulatory cofactor NHE-RF1	↓
Q60928	Glutathione hydrolase 1 proenzyme	↓
P52760	2-iminobutanoate/2-iminopropanoate deaminase	↓
Q91ZI0	Cadherin EGF LAG seven-pass G-type receptor 3	↓
Q9JI02	Secretoglobin family 2B member 20	↓
Q60932	Voltage-dependent anion-selective channel protein 1	↓
P12658	Calbindin	↑
P99029	Peroxiredoxin-5, mitochondrial	↑

通过 Uniprot 检索蛋白的功能和涉及的生物学过程，其中 Na(+)/H(+) exchange regulatory cofactor NHE-RF1 具有多巴胺受体结合、γ-氨基丁酸跨膜转运等功能，参与到腺苷酸环化酶激活多巴胺受体信号通路、磷脂酶 c 激活多巴胺受体信号通路等生物学过程中；Cadherin EGF LAG seven-pass G-type receptor 3 是神经系统形成过程中，在细胞间信号传导中起重要作用的受体；参与到轴突束颤、多巴胺能神经元轴突引导、运动神经元迁移、平面细胞极性通路参与轴突引导、5 -羟色胺能神经元轴突引导等生物学过程中；Calbindin 在轴突、树突、树突棘、gamma-aminobutyric acid 能突触、谷氨酸能突触、神经元胞体等区域中表达，参与到突触可塑性调控、通过钙离子结合调节突触前后胞质钙离子浓度、突触前胞质钙离子浓度的调节、长期记忆、短期记忆等生物学过程中。这可能与甜味觉的喜好反应和脑奖励回路相关。Glutathione hydrolase 1 proenzyme 具有谷胱甘肽水解酶活性，参与谷胱甘肽的分解代谢、谷氨酸的代谢过程和对酒精的反应。

同时对单个小鼠食用安赛蜜后前的尿液蛋白质组差异蛋白进行检索，查找单个小鼠尿液蛋白质组差异蛋白中具有的脑奖励回路相关蛋白，如表 9 所示。

表 9 安赛蜜组个体食用安赛蜜后前尿液蛋白质组差异蛋白中与脑奖励回路相关的蛋白

	glutamate	dopamine	gamma-aminobutyric acid / GABA	Substance P	enkephalin	Other neural correlations
11	Cadherin-2 Na(+)/H(+) exchange regulatory cofactor NHE-RF1 Glutathione hydrolase 1 proenzyme Calbindin Calmodulin-1 Glyceraldehyde-3-phosphate dehydrogenase	Na(+)/H(+) exchange regulatory cofactor NHE-RF1 Cadherin EGF LAG seven-pass G-type receptor 3	Calbindin Na(+)/H(+) exchange regulatory cofactor NHE-RF1			Voltage-dependent anion-selective channel protein 1 Ganglioside GM2 activator
12	Ephrin type-B receptor 2 Aggrecan core protein 14-3-3 protein zeta/delta Na(+)/H(+) exchange regulatory cofactor NHE-RF1 Glutathione hydrolase 1 proenzyme Calbindin Calmodulin-1 Amino acid transporter heavy chain SLC3A1 Frizzled-4	Na(+)/H(+) exchange regulatory cofactor NHE-RF1 Cadherin EGF LAG seven-pass G-type receptor 3	Aggrecan core protein Cadherin-13 Calbindin Cathepsin D Na(+)/H(+) exchange regulatory cofactor NHE-RF1			Alpha-L-iduronidase Legumain Beta-2-microglobulin Voltage-dependent anion-selective channel protein 1 Ganglioside GM2 activator Angiotensinogen
13	Aspartate aminotransferase, cytoplasmic Progranulin Neuroplastin Interleukin-1 receptor accessory protein Profilin-1 Na(+)/H(+) exchange regulatory cofactor NHE-RF1 Glutathione hydrolase 1 proenzyme Calbindin Amino acid transporter heavy chain SLC3A1 Frizzled-4	Na(+)/H(+) exchange regulatory cofactor NHE-RF1 Cadherin EGF LAG seven-pass G-type receptor 3	Neuroplastin Calbindin Na(+)/H(+) exchange regulatory cofactor NHE-RF1	Enkephalinase	Enkephalinase	Voltage-dependent anion-selective channel protein 1
14	Aspartate aminotransferase, cytoplasmic Microtubule-associated protein 2 Na(+)/H(+) exchange regulatory cofactor NHE-RF1 Glutathione hydrolase 1 proenzyme Calbindin Calmodulin-1	Stromal cell-derived factor 1 Na(+)/H(+) exchange regulatory cofactor NHE-RF1 Cadherin EGF LAG seven-pass G-type receptor 3 Sepiapterin reductase	Calbindin Cathepsin D Na(+)/H(+) exchange regulatory cofactor NHE-RF1			Beta-2-microglobulin Voltage-dependent anion-selective channel protein 1
15	Fractalkine Aspartate aminotransferase, cytoplasmic Ephrin type-B receptor 2 Progranulin Neuroplastin Glypican-4 Amino acid transporter heavy chain SLC3A2 Syntenin-1 Neuronal membrane glycoprotein M6-a Na(+)/H(+) exchange regulatory cofactor NHE-RF1 Glutathione hydrolase 1 proenzyme Calbindin Amino acid transporter heavy chain SLC3A1 Frizzled-4	Na(+)/H(+) exchange regulatory cofactor NHE-RF1 Cadherin EGF LAG seven-pass G-type receptor 3 Sepiapterin reductase	Neuroplastin Ephrin-A5 Carbonic anhydrase 2 Calbindin Na(+)/H(+) exchange regulatory cofactor NHE-RF1			Alpha-L-iduronidase Voltage-dependent anion-selective channel protein 1

3.7 三氯蔗糖组尿液蛋白质组成组分析

3.7.1 差异蛋白

三氯蔗糖实验组与三氯蔗糖对照组尿液蛋白质进行比较，筛选差异蛋白条件为：FC≥1.5 或≤0.67，双尾非配对 t 检验 P<0.05。结果表明，三氯蔗糖实验组与三氯蔗糖对照组相比，可以鉴定到 83 个差异蛋白，将差异蛋白按 FC 由小到大的顺序排列，通过 Uniprot 进行检索，结果如表 10 所示。

表 10 三氯蔗糖实验组与三氯蔗糖对照组小鼠尿液蛋白质组差异蛋白

Uniprot ID	Protein names	Fold change	Trend	P value
P08905	Lysozyme C-2	0.054	↓	4.56E-02
Q8R1U2	Cell growth regulator with EF hand domain protein 1	0.099	↓	2.29E-02
P70441	Na(+)/H(+) exchange regulatory cofactor NHE-RF1	0.136	↓	9.18E-03
P51655	Glypican-4	0.155	↓	2.69E-02
Q923L3	CUB and sushi domain-containing protein 1	0.176	↓	4.58E-02
P41272	CD27 antigen	0.181	↓	4.68E-03
Q02596	Glycosylation-dependent cell adhesion molecule 1	0.226	↓	1.53E-02
Q91WZ8	Dysbindin	0.244	↓	1.16E-03

P19221	Prothrombin	0.245	↓	4.84E-02
P47878	Insulin-like growth factor-binding protein 3	0.252	↓	4.50E-02
P10923	Osteopontin	0.255	↓	3.60E-02
Q9JJS0	Signal peptide, CUB and EGF-like domain-containing protein 2	0.262	↓	3.60E-02
P11087	Collagen alpha-1(I) chain	0.270	↓	6.89E-03
Q9JHY3	WAP four-disulfide core domain protein 12	0.273	↓	2.37E-02
O35188	Fractalkine	0.282	↓	2.16E-02
O08810	116 kDa U5 small nuclear ribonucleoprotein component	0.300	↓	1.54E-02
Q61592	Growth arrest-specific protein 6	0.331	↓	4.47E-04
P04441	H-2 class II histocompatibility antigen gamma chain	0.346	↓	3.83E-02
Q60928	Glutathione hydrolase 1 proenzyme	0.358	↓	5.94E-03
P04186	Complement factor B	0.368	↓	1.18E-02
Q9ET66	Peptidase inhibitor 16	0.374	↓	3.46E-02
Q91WV7	Neutral and basic amino acid transport protein rBAT	0.377	↓	3.91E-03
O88307	Sortilin-related receptor	0.387	↓	4.66E-02
P61110	Kidney androgen-regulated protein	0.387	↓	7.18E-03
P08228	Superoxide dismutase [Cu-Zn]	0.421	↓	4.93E-02
P25119	Tumor necrosis factor receptor superfamily member 1B	0.426	↓	3.70E-02
Q99P86	Resistin-like beta	0.429	↓	3.86E-02
Q9DAK9	14 kDa phosphohistidine phosphatase	0.430	↓	1.21E-02
P12023	Amyloid-beta precursor protein	0.434	↓	1.60E-02
Q9WTR5	Cadherin-13	0.439	↓	4.16E-02
Q6ZWQ0	Nesprin-2	0.441	↓	2.18E-03
B2RPV6	Multimerin-1	0.449	↓	4.45E-02
Q6PDN3	Myosin light chain kinase, smooth muscle	0.457	↓	2.73E-02
Q91XE4	N-acyl-aromatic-L-amino acid amidohydrolase (carboxylate-forming)	0.462	↓	5.55E-03
D3YXG0	Hemicentin-1	0.464	↓	2.37E-02
B5X0G2	Major urinary protein 17	0.477	↓	6.00E-04
P11588	Major urinary protein 1	0.481	↓	3.28E-02
P03953	Complement factor D	0.486	↓	1.95E-02
Q60823	RAC-beta serine/threonine-protein kinase	0.502	↓	3.15E-02
Q9DBH5	Vesicular integral-membrane protein VIP36	0.509	↓	1.24E-02
O89017	Legumain	0.517	↓	1.63E-02
Q9R1P3	Proteasome subunit beta type-2	0.527	↓	6.43E-04
Q9Z319	Atrial natriuretic peptide-converting enzyme	0.536	↓	1.14E-02
P55288	Cadherin-11	0.538	↓	3.26E-02
P10493	Nidogen-1	0.567	↓	2.60E-02
Q61090	Frizzled-7	0.575	↓	3.87E-02
P97808	FXYD domain-containing ion transport regulator 5	0.588	↓	1.20E-02
P04939	Major urinary protein 3	0.597	↓	4.10E-03
A2AEP0	Odorant-binding protein 1b	0.607	↓	3.67E-02
A2BIM8	Major urinary protein 18	0.637	↓	2.91E-02

Q921I1	Serotransferrin	1.855	↑	7.87E-03
P23953	Carboxylesterase 1C	1.880	↑	1.36E-02
P32261	Antithrombin-III	2.585	↑	4.62E-02
P60710	Actin, cytoplasmic 1	2.742	↑	2.18E-02
P63017	Heat shock cognate 71 kDa protein	3.118	↑	2.94E-02
P35700	Peroxiredoxin-1	3.312	↑	1.87E-02
P63101	14-3-3 protein zeta/delta	3.670	↑	2.58E-02
Q64726	Zinc-alpha-2-glycoprotein	3.920	↑	1.43E-02
Q61147	Ceruloplasmin	3.949	↑	3.85E-03
P05064	Fructose-bisphosphate aldolase A	4.140	↑	4.77E-02
Q9DBJ1	Phosphoglycerate mutase 1	4.655	↑	4.64E-02
Q8VCS0	N-acetylmuramoyl-L-alanine amidase	5.285	↑	4.49E-02
P12658	Calbindin	5.638	↑	2.99E-02
P16125	L-lactate dehydrogenase B chain	5.825	↑	3.17E-02
P62737	Actin, aortic smooth muscle	5.945	↑	4.11E-02
P28665	Murinoglobulin-1	7.723	↑	2.08E-02
Q00724	Retinol-binding protein 4	8.998	↑	4.76E-02
Q9ESB3	Histidine-rich glycoprotein	10.513	↑	4.93E-02
P05201	Aspartate aminotransferase, cytoplasmic	12.205	↑	9.78E-03
P17182	Alpha-enolase	13.961	↑	2.53E-02
Q01768	Nucleoside diphosphate kinase B	16.303	↑	9.79E-03
P45376	Aldo-keto reductase family 1 member B1	20.342	↑	2.83E-02
Q9DCG2	CD302 antigen	21.131	↑	2.48E-02
P56480	ATP synthase subunit beta, mitochondrial	23.226	↑	2.28E-02
P30275	Creatine kinase U-type, mitochondrial	23.354	↑	4.79E-02
P52480	Pyruvate kinase PKM	29.633	↑	4.65E-03
O70250	Phosphoglycerate mutase 2	30.187	↑	3.34E-02
P50543	Protein S100-A11	41.961	↑	1.85E-02
P14152	Malate dehydrogenase, cytoplasmic	45.174	↑	4.45E-02
P05784	Keratin, type I cytoskeletal 18	48.213	↑	6.27E-04
P62962	Profilin-1	2930.285	↑	2.66E-02
P09411	Phosphoglycerate kinase 1	从无到有	↑	2.75E-05
P50516	V-type proton ATPase catalytic subunit A	从无到有	↑	2.36E-02

3.7.2 差异蛋白功能分析

将鉴定到的 83 个差异蛋白经过 DAVID 数据库进行分子功能和生物学过程分析，结果如图 8 所示。

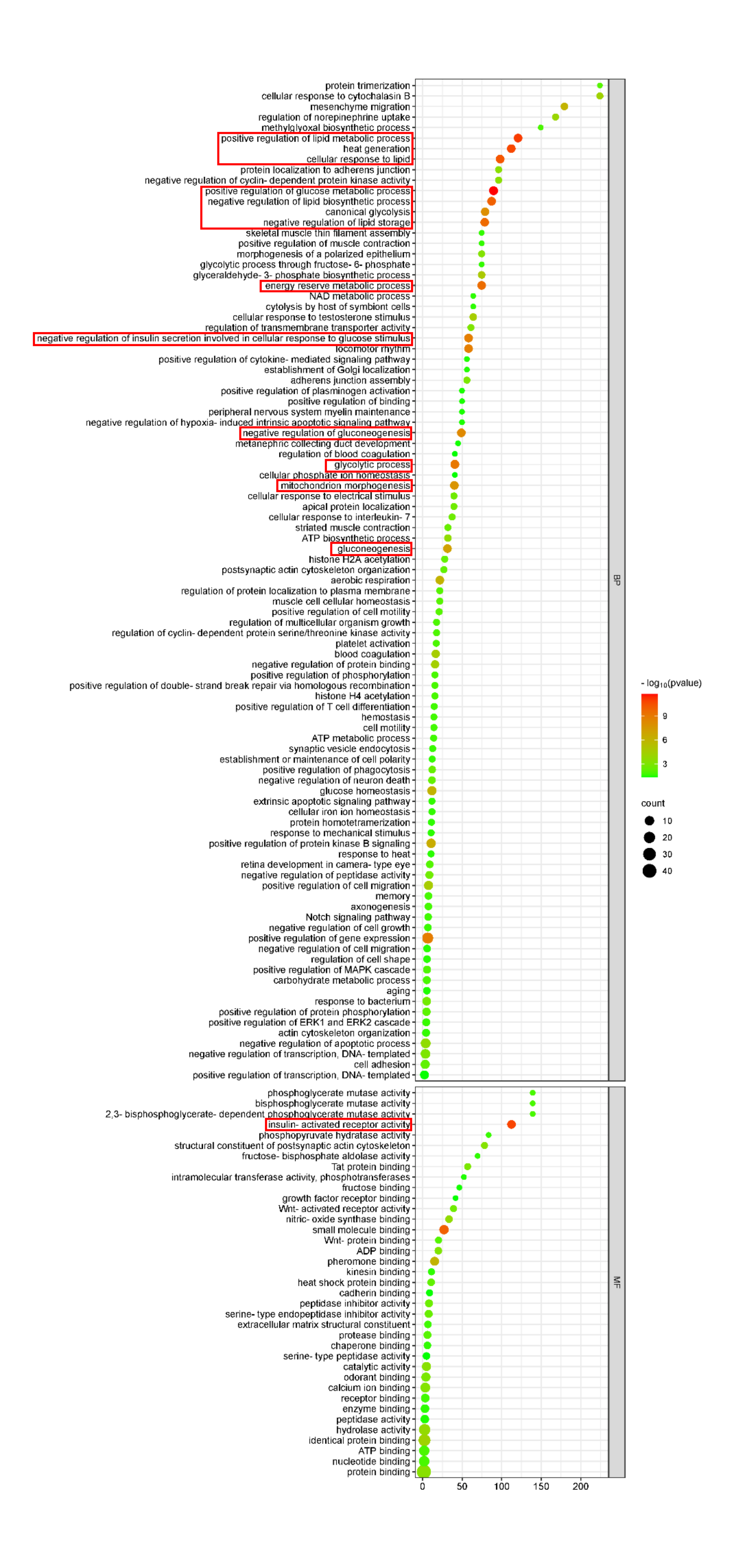


图 8 三氯蔗糖组小鼠差异蛋白分子功能和生物学过程分析

按 p 值由小到大排序，其中排名前 30%的 28 个生物学过程中，有 16 个生物学过程涉及到了大量糖、脂代谢和能量生产，如：葡萄糖代谢过程的正向调节、正向调节脂质代谢过程、热的产生、细胞对脂质的反应、脂质生物合成过程的负调控、负向调节脂质储存、能量储备代谢过程、糖酵解过程、胰岛素分泌的负调控参与细胞对葡萄糖刺激的反应等，与蔗糖组高度相似；分子功能也与蔗糖组相似，按 p 值由小到大排序，其中排名第一的分子功能是胰岛素受体激活。值得注意的是，差异蛋白同样富集到了神经元死亡负向调控、轴突的发生、突触后肌动蛋白细胞骨架组织、突触囊泡内吞作用、外周神经系统髓磷脂维持等神经相关的生物学过程，多于蔗糖、甜菊糖苷和安赛蜜，这可能与甜味觉的喜好反应相关。

3.7.3 差异蛋白与脑奖励回路

为了进一步探究尿液蛋白质组差异蛋白是否有脑奖励回路相关的蛋白，通过 Uniprot 检索三氯蔗糖组差异蛋白的功能和涉及的生物学过程，寻找是否与已报道的脑奖励回路中的关键蛋白相关，结果如表 11 所示。

表 11 三氯蔗糖组小鼠尿液蛋白质组差异蛋白与脑奖励回路

The Brain’s Reward Circuitry	
Glutamate	√
Dopamine	√
Gamma-aminobutyric acid / GABA	√
Substance P	
Enkephalin	

小鼠尿液蛋白质组差异蛋白中检索到有和脑奖励回路相关的蛋白。

Na(+)/H(+) exchange regulatory cofactor NHE-RF1 具有多巴胺受体结合、γ-氨基丁酸跨膜转运等功能，参与到腺苷酸环化酶激活多巴胺受体信号通路、磷脂酶 c 激活多巴胺受体信号通路等生物学过程中。

Glypican-4 位于谷氨酸能突触、突触及前膜等区域表达，能参与中枢神经系统的发育，参与到神经递质受体定位对突触后特化膜的调控、突触前组装的调控、突触膜粘附等生物学过程中。

Dysbindin 位于不对称轴突、轴突细胞质、树突棘、神经元胞体、突触后膜等区域表达，具有突触囊泡运输、神经递质释放、神经突生长和影响谷氨酸能释放来促进神经元的传递和活力的功能，参与到顺行轴突转运、顺行突触囊泡转运、树突形态发生、谷氨酸神经递质的分泌、多巴胺受体信号通路的调控、多巴胺分泌调节、突触小泡胞吐调节等生物学过程中。

Fractalkine 位于神经元胞体等区域表达，参与到抑制性突触后电位、谷氨酸受体信号通路的负调控海马神经元凋亡过程的负调控、神经元细胞稳态、神经母细胞增殖正向调节等生物学过程中。

Glutathione hydrolase 1 proenzyme 具有谷胱甘肽水解酶活性，参与谷胱甘肽的分解代谢、谷氨酸的代谢过程和对酒精的反应。Amyloid-beta precursor protein 作为细胞表面受体，在神经元表面执行与神经突生长、神经元粘附和轴突发生有关的生理功能；参与学习和记忆、谷氨酸受体信号通路、联想学习、神经元分化等生物学过程中。

Amino acid transporter heavy chain SLC3A1 参与到谷氨酸的跨膜转运。

Amyloid-beta precursor protein 作为细胞表面受体，在神经元表面执行与神经突生长、神经元粘附和轴突发生有关的生理功能；参与学习和记忆、谷氨酸受体信号通路、联想学习、神经元分化等生物学过程中。

Cadherin-13 在 gamma-aminobutyric acid 能突触、神经投射等区域表达，参与到钙介导的信号通路正向调节等生物学过程中

Cadherin-11 在谷氨酸能突触等区域中表达，参与到皮质脊髓束形态发生、化学突触传递的调节等生物学过程中。

Heat shock cognate 71 kDa protein 在不对称轴突、轴突、树突和树突棘、谷氨酸能突触、髓鞘、突触后膜、突触囊泡等区域表达，参与到突触后组织的调控、对气味的反应、慢轴突转运等生物学过程中。

14-3-3 protein zeta/delta 在谷氨酸能突触等区域表达，参与到突触成熟的调控、突触目标的识别等生物学过程中。

Calbindin 在轴突、树突、树突棘、gamma-aminobutyric acid 能突触、谷氨酸能突触、神经元胞体等区域中表达，参与到突触可塑性调控、通过钙离子结合调节突触前后胞质钙离子浓度、突触前胞质钙离子浓度的调节、长期记忆、短期记忆等生物学过程中。

Aspartate aminotransferase, cytoplasmic 能催化合成谷氨酸：脊椎动物中枢神经系统的主要兴奋性神经递质，是谷氨酸水平的重要调节剂。在脑神经保护中起谷氨酸清除剂的作用。参与到谷氨酸合成和分解代谢等生物学过程中。

Profilin-1 在谷氨酸能突触、突触前后细胞等区域表达，参与到化学突触传递的调节、突触成熟等生物学过程中。

3.8 三氯蔗糖组个体食用三氯蔗糖后前尿液蛋白质组单个分析

分别将 5 只小鼠食用三氯蔗糖后的尿液蛋白质组与食用三氯蔗糖前的尿液蛋白质组进行比较，筛选差异蛋白条件为：FC≥1.5 或≤0.67，双尾非配对 t 检验 P<0.05，统计 5 只小鼠共有的差异蛋白，结果如图 9 所示。

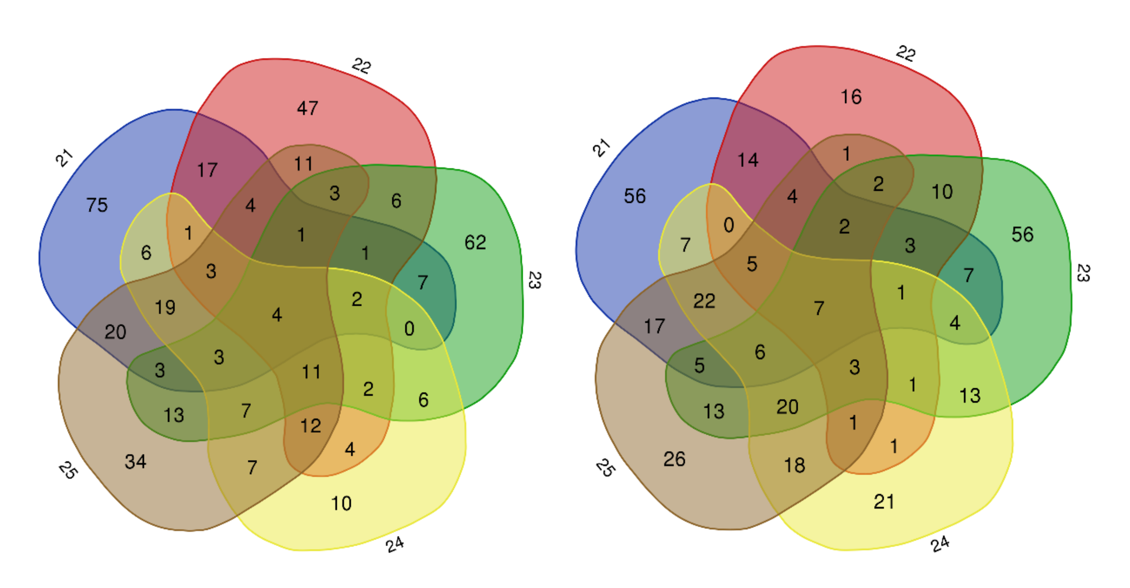


图 9 三氯蔗糖组个体食用三氯蔗糖后前共有上调差异蛋白（左）下调差异蛋白（右）

5 只小鼠共有 4 个上调差异蛋白，12 个下调差异蛋白，如表 12 所示。

表 12 三氯蔗糖组个体食用三氯蔗糖后前共有差异蛋白

Uniprot ID	Protein name	Trend
P05784	Keratin, type I cytoskeletal 18	↓
P10923	Osteopontin (2AR)	↓
P41272	CD27 antigen	↓
P15626	Glutathione S-transferase Mu 2	↓
P04939	Major urinary protein 3	↓
P70441	Na(+)/H(+) exchange regulatory cofactor NHE-RF1	↓
Q60928	Glutathione hydrolase 1 proenzyme	↓
P11087	Collagen alpha-1(I) chain	↑
P09411	Phosphoglycerate kinase 1	↑
P12658	Calbindin	↑
Q02596	Glycosylation-dependent cell adhesion molecule 1	↑

通过 Uniprot 检索蛋白的功能和涉及的生物学过程，其中 Na(+)/H(+) exchange regulatory cofactor NHE-RF1 具有多巴胺受体结合、γ-氨基丁酸跨膜转运等功能，参与到腺苷酸环化酶激活多巴胺受体信号通路、磷脂酶 c 激活多巴胺受体信号通路等生物学过程中；Calbindin 在轴突、树突、树突棘、gamma-aminobutyric acid 能突触、谷氨酸能突触、神经元胞体等区域中表达，参与到突触可塑性调控、通过钙离子结合调节突触前后胞质钙离子浓度、突触前胞质钙离子浓度的调节、长期记忆、短期记忆等生物学过程中。这可能与甜味觉的喜好反应和脑奖励回路相关。Glutathione hydrolase 1 proenzyme 具有谷胱甘肽水解酶活性，参与谷胱甘肽的分解代谢、谷氨酸的代谢过程和对酒精的反应。

同时对单个小鼠食用三氯蔗糖后前的尿液蛋白质组差异蛋白进行检索，查找单个小鼠尿液蛋白质组差异蛋白中具有的脑奖励回路相关蛋白，如表 13 所示。

表 13 三氯蔗糖组个体食用三氯蔗糖后前尿液蛋白质组差异蛋白中与脑奖励回路相关的蛋白

	Glutamate	Dopamine	Gamma-aminobutyric acid / GABA	Substance P	Enkephalin	Other neural correlations
21	Calbindin Aspartate aminotransferase, cytoplasmic 14-3-3 protein zeta/delta Glyceraldehyde-3-phosphate dehydrogenase Carboxypeptidase Q Interleukin-1 receptor accessory protein Gamma-glutamyl hydrolase Angiotensin-converting enzyme Calmodulin-1 Epidermal growth factor receptor Na(+)/H(+) exchange regulatory cofactor NHE-RF1 Glutathione hydrolase 1 proenzyme Cadherin-11 Amino acid transporter heavy chain SLC3A1 Myc box-dependent-interacting protein 1 Cadherin-1 Progranulin Amyloid-beta precursor protein Palmitoyltransferase ZDHHC8 Glypican-4	Cadherin EGF LAG seven-pass G-type receptor 3 Palmitoyltransferase ZDHHC8 Na(+)/H(+) exchange regulatory cofactor NHE-RF1	Na(+)/H(+) exchange regulatory cofactor NHE-RF1 Calbindin Carbonic anhydrase 2 Cadherin-13	Angiotensin-converting enzyme	Angiotensin-converting enzyme Procathepsin L	Angiotensinogen Ganglioside GM2 activator Beta-2-microglobulin Legumain
22	Calbindin Carboxypeptidase Q Interleukin-1 receptor accessory protein Transitional endoplasmic reticulum ATPase Cofilin-1 Heat shock cognate 71 kDa protein Profilin-1 Progranulin Angiotensin-converting enzyme Na(+)/H(+) exchange regulatory cofactor NHE-RF1 Glutathione hydrolase 1 proenzyme Cadherin-11 Amino acid transporter heavy chain SLC3A1	Parkinson disease protein 7 homolog Na(+)/H(+) exchange regulatory cofactor NHE-RF1	Na(+)/H(+) exchange regulatory cofactor NHE-RF1 Calbindin Carbonic anhydrase 2	Angiotensin-converting enzyme	Procathepsin L Angiotensin-converting enzyme	Beta-2-microglobulin Voltage-dependent anion-selective channel protein 1 Ganglioside GM2 activator

23	Calbindin Aspartate aminotransferase, cytoplasmic 14-3-3 protein zeta/delta Transitional endoplasmic reticulum ATPase Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial Dystroglycan 1 Amino acid transporter heavy chain SLC3A2 Ornithine aminotransferase, mitochondrial Gamma-glutamyl hydrolase Carboxypeptidase Q Interleukin-1 receptor accessory protein Epidermal growth factor receptor SPARC-like protein 1 Na(+)/H(+) exchange regulatory cofactor NHE-RF1 Glutathione hydrolase 1 proenzyme Amino acid transporter heavy chain SLC3A1 Myc box-dependent-interacting protein 1 Cadherin-1 Cadherin-2 Plasminogen	Sepiapterin reductase Aldehyde dehydrogenase, mitochondrial Cadherin EGF LAG seven-pass G-type receptor 3 Na(+)/H(+) exchange regulatory cofactor NHE-RF1	Na(+)/H(+) exchange regulatory cofactor NHE-RF1 Dystroglycan 1 Calbindin			Beta-2-microglobulin Glucose-6-phosphate isomerase ATP-binding cassette sub-family A member 7 Voltage-dependent anion-selective channel protein 1 Angiotensinogen
24	Calbindin Aspartate aminotransferase, cytoplasmic 14-3-3 protein zeta/delta Glyceraldehyde-3-phosphate dehydrogenase Semaphorin-4B Na(+)/H(+) exchange regulatory cofactor NHE-RF1 Glutathione hydrolase 1 proenzyme Cadherin-11 Myc box-dependent-interacting protein 1 Progranulin Fractalkine	Parkinson disease protein 7 homolog Na(+)/H(+) exchange regulatory cofactor NHE-RF1	Na(+)/H(+) exchange regulatory cofactor NHE-RF1 Calbindin Carbonic anhydrase 2 Cadherin-13			ATP-binding cassette sub-family A member 7 Voltage-dependent anion-selective channel protein 1
25	Calbindin Aspartate aminotransferase, cytoplasmic 14-3-3 protein zeta/delta Glyceraldehyde-3-phosphate dehydrogenase Gamma-glutamyl hydrolase Angiotensin-converting enzyme Cofilin-1 Heat shock cognate 71 kDa protein Receptor-type tyrosine-protein phosphatase delta Syntenin-1 Neuroplastin Calmodulin-1 Na(+)/H(+) exchange regulatory cofactor NHE-RF1 Glutathione hydrolase 1 proenzyme Cadherin-11 Cadherin-1 Progranulin Amyloid-beta precursor protein Cadherin-2 Plasminogen Fractalkine	Parkinson disease protein 7 homolog Cadherin EGF LAG seven-pass G-type receptor 3 Na(+)/H(+) exchange regulatory cofactor NHE-RF1	Na(+)/H(+) exchange regulatory cofactor NHE-RF1 Calbindin Carbonic anhydrase 2 Cadherin-13 Neuroplastin	Angiotensin-converting enzyme	Angiotensin-converting enzyme	Lysosomal alpha-mannosidase Legumain

4 甜味物质的横向比较分析

4.1 成组分析差异蛋白的比较

成组分析中 4 种甜味物质的共有差异蛋白，如图 10 所示。

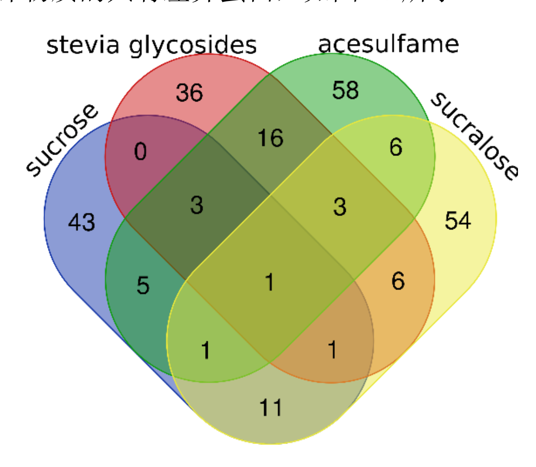


图 10 4 种甜味物质成组分析的共有差异蛋白

与蔗糖组相比，甜菊糖苷组与蔗糖组共有 5 个差异蛋白，安赛蜜组与蔗糖组共有 10 个差异蛋白，三氯蔗糖组与蔗糖组共有 15 个差异蛋白。与甜菊糖苷相比，安赛蜜组与甜菊糖苷组共有 23 个差异蛋白，三氯蔗糖组与甜菊糖苷组共有 11 个差异蛋白。与安赛蜜相比，

三氯蔗糖组与安赛蜜组共有 11 个差异蛋白。其中安赛蜜组与甜菊糖苷组成组分析时，共有差异蛋白最多，其次是三氯蔗糖组与蔗糖组，甜菊糖苷组与蔗糖组成组分析时，共有差异蛋白最少。具体蛋白见附表 1。

成组分析中 4 种甜味物质的共有生物学过程，如图 11 所示。

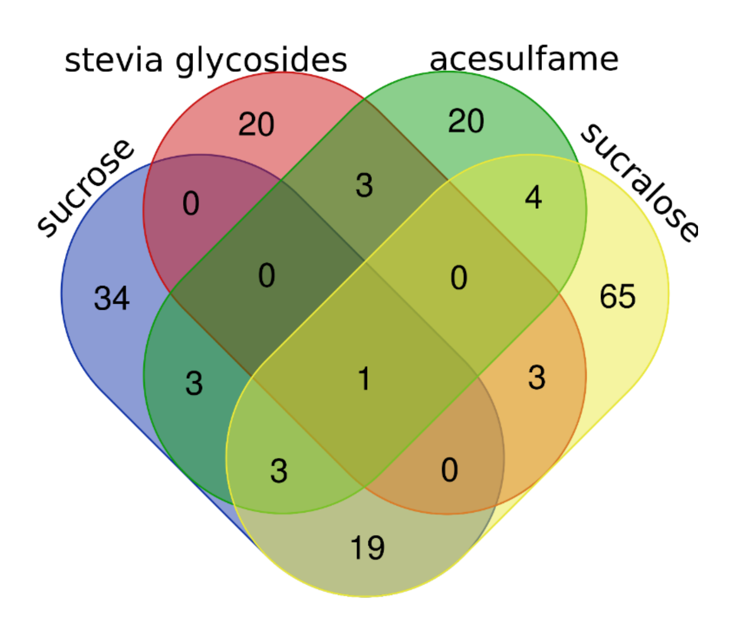


图 11 4 种甜味物质成组分析的共有生物学过程

与蔗糖组相比，甜菊糖苷组与蔗糖组共有 1 个生物学过程，安赛蜜组与蔗糖组共有 7 个生物学过程，三氯蔗糖组与蔗糖组共有 23 个生物学过程。与甜菊糖苷相比，安赛蜜组与甜菊糖苷组共有 4 个生物学过程，三氯蔗糖组与甜菊糖苷组共有 4 个生物学过程。与安赛蜜相比，三氯蔗糖组与安赛蜜组共有 8 个生物学过程。其中三氯蔗糖组与蔗糖组成组分析时，共有生物学过程最多，且远高于其他组；甜菊糖苷组与蔗糖组成组分析时，共有差异蛋白最少。值得注意的是，甜菊糖苷组与其他甜味物质的共有生物学过程也少，这可能说明甜菊糖苷对机体的影响与其他甜味剂不同。具体生物学过程见附表 2。

4 种甜味物质组差异蛋白富集的生物学过程均与神经系统相关，虽然所涉及的具体生物学过程不同，且变化并不是所有生物学过程中最显著的，但这很可能与甜味觉的喜好反应相关；蔗糖组、安赛蜜组和三氯蔗糖组差异蛋白富集的生物学过程中主要涉及糖脂代谢、能量的产生等，很少有核小体组装、基因表达相关的变化，而甜菊糖苷组差异蛋白富集的生物学过程中主要涉及的是核小体组装、基因表达、细胞分裂等，很少有能量代谢相关的变化，这很可能说明甜菊糖苷食用后引起的机体反应与其他三种甜味物质不同。

成组分析中 4 种甜味物质的共有差异蛋白分子功能，如图 12 所示。

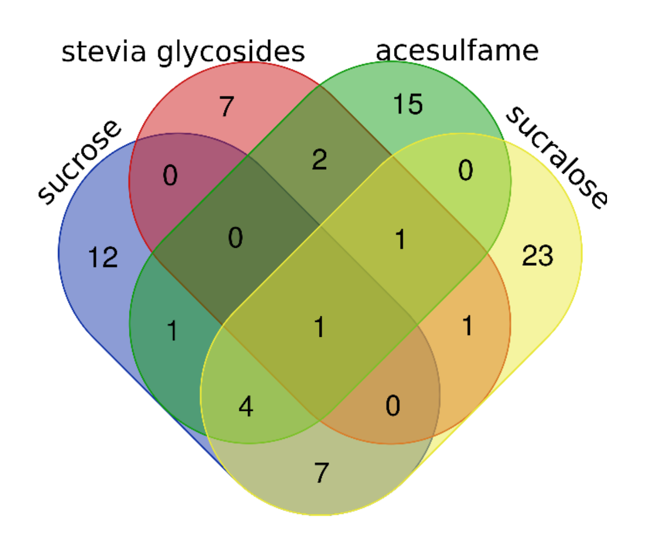


图 12 4 种甜味物质成组分析的共有差异蛋白分子功能

与蔗糖组相比，甜菊糖苷组与蔗糖组共有 1 个共有差异蛋白分子功能，安赛蜜组与蔗糖组共有 4 个共有差异蛋白分子功能，三氯蔗糖组与蔗糖组共有 12 个共有差异蛋白分子功能。与甜菊糖苷相比，安赛蜜组与甜菊糖苷组共有 4 个共有差异蛋白分子功能，三氯蔗糖组与甜菊糖苷组共有 3 个共有差异蛋白分子功能。与安赛蜜相比，三氯蔗糖组与安赛蜜组共有 6 个共有差异蛋白分子功能。其中三氯蔗糖组与蔗糖苷组成组分析时，共有差异蛋白分子功能最多，且远高于其他组，甜菊糖苷与蔗糖组成组分析时，共有差异蛋白分子功能最少。值得注意的是，甜菊糖苷组与其他甜味物质的共有差异蛋白分子功能最少。具体生物学过程见附表 3。

蔗糖组和三氯蔗糖组差异蛋白的分子功能中显著出现和血糖变化直接相关的胰岛素受体激活，而甜菊糖苷和安赛蜜组均未出现，这可能说明，三氯蔗糖食用后相对于甜菊糖苷和安赛蜜，更容易像蔗糖一样引起血糖波动。

成组分析中 4 种甜味物质组差异蛋白均包含和脑奖励回路过程相关的蛋白，如表 14 所示。

表 14 4 种甜味物质成组分析中与脑奖励回路的差异蛋白

	Glutamate	Gopamine	Gamma-aminobutyric acid / GABA	Substance P	Enkephalin	Other neural correlations
sucrose	Amyloid-beta precursor protein		Acyl-CoA-binding protein	Enkephalinase	Procathepsin L	Angiotensinogen
	14-3-3 protein zeta/delta		Carbonic anhydrase 2		Enkephalinase	
stevia glycosides	Frizzled	Sepiapterin reductase	Na(+)/H(+) exchange regulatory cofactor NHE-RF1			
	Glypican-4	Na(+)/H(+) exchange regulatory cofactor NHE-RF1				
	Tyrosine-protein kinase transmembrane receptor ROR2					
	Glutathione hydrolase 1 proenzyme					
acesulfame	Neuronal membrane glycoprotein M6-a	Na(+)/H(+) exchange regulatory cofactor NHE-RF1	Na(+)/H(+) exchange regulatory cofactor NHE-RF1			Ephrin type-B receptor 2
	Glutathione hydrolase 1 proenzyme					
	Syntenin-1					
	Amino acid transporter heavy chain SLC3A1	Cadherin EGF LAG seven-pass G-type receptor 3	Calbindin			
sucralose	Glypican-4	Dysbindin	Calbindin			
	Fractalkine	Na(+)/H(+) exchange regulatory cofactor NHE-RF1	Cadherin-13			
	Aspartate aminotransferase, cytoplasmic					
	Profilin-1					
	Amyloid-beta precursor protein					
	Cadherin-11					
	Heat shock cognate 71 kDa protein					
	14-3-3 protein zeta/delta					
	Amino acid transporter heavy chain SLC3A1					
	Glutathione hydrolase 1 proenzyme					

其中蔗糖组和三氯蔗糖组涉及的脑奖励回路相关蛋白种类较其他组更多，这可能说明蔗糖和三氯蔗糖引起的脑奖励回路更加复杂，成瘾性也可能更强。

4 种甜味物质组差异蛋白均未出现和甜味觉感知相关的蛋白，推测可能是甜味觉感知

的蛋白并不会出现在尿液蛋白质组中，但是尿液蛋白质组差异蛋白中出现了与脑奖励回路相关的蛋白。

根据以上成组分析的结果初步推断，4 种甜味物质中，三氯蔗糖与蔗糖引起机体的变化最为相似，甜菊糖苷与蔗糖引起机体的变化相差最远，且甜菊糖苷引起机体的变化与其他甜味物质不同。4 种甜味物质均能引起尿液蛋白质组差异蛋白出现与脑奖励回路相关的蛋白，而只有蔗糖、安赛蜜和三氯蔗糖能引起尿液蛋白质组中大量出现代谢过程变化。

4.1 单个分析共有差异蛋白的比较

单个分析中 4 种甜味物质共有的共有差异蛋白，如图 13 所示。

单个分析时，蔗糖组仅有和脑奖励回路相关的共有差异蛋白，甜菊糖苷组有和 mRNA 代谢、脑奖励回路相关的共有差异蛋白；安赛蜜和三氯蔗糖组的共有的差异蛋白主要是各种水解酶，参与到代谢过程中，同时也有脑奖励回路相关的共有差异蛋白。

与蔗糖组相比，甜菊糖苷组与蔗糖组共有 0 个共有差异蛋白，安赛蜜组与蔗糖组共有 0 个共有差异蛋白，三氯蔗糖组与蔗糖组共有 0 个共有差异蛋白。与甜菊糖苷相比，安赛蜜组与甜菊糖苷组共有 2 个共有差异蛋白，三氯蔗糖组与甜菊糖苷组共有 1 个共有差异蛋白。与安赛蜜相比，三氯蔗糖组与安赛蜜组共有 2 个共有差异蛋白。其中三氯蔗糖组与安赛蜜组单个分析时，共有的共有差异蛋白最多，且 2 个共有差异蛋白与脑奖励回路相关，这两个蛋白的变化趋势也相同。具体共有差异蛋白见附表 4。

这些结果可能说明了，4 种甜味物质导致的尿液蛋白质组差异蛋白中出现和脑奖励回路相关的蛋白，在不同个体之间是相对稳定存在的。其中蔗糖、安赛蜜和三氯蔗糖导致的尿液蛋白质组差异蛋白中出现和糖脂代谢等相关的蛋白，在蔗糖组不同个体之间不是相对稳定存在的，而在安赛蜜和三氯蔗糖组不同个体之间不是相对稳定存在的。

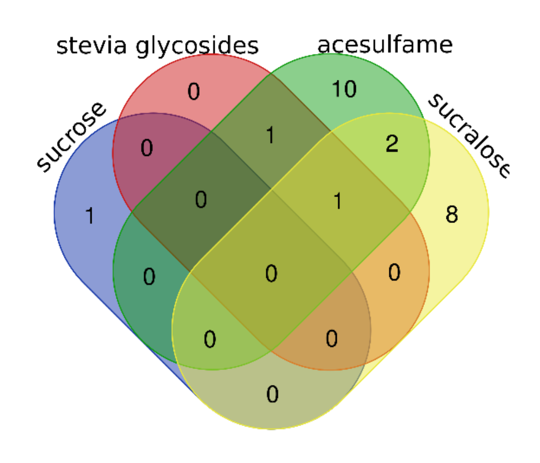


图 13 4 种甜味物质单个分析共有的共有差异蛋白

5 总结

成组分析小鼠自主食用蔗糖后前尿液蛋白质组差异蛋白及其富集的生物学过程，大部分与糖脂代谢相关，也有小部分和神经系统相关的生物学过程，差异蛋白中有和脑奖励回路相关的蛋白，但没有和甜味觉感知相关的蛋白；单只分析小鼠自主食用蔗糖后前尿液蛋白质组的共有差异蛋白，具有与脑奖励回路相关的蛋白。

成组分析小鼠自主食用甜菊糖苷后前尿液蛋白质组差异蛋白及其富集的生物学过程，大部分与核小体组装、基因表达、细胞分裂相关，也有小部分和神经系统相关的生物学过程，差异蛋白中有和脑奖励回路相关的蛋白，但没有和甜味觉感知相关的蛋白；单只分析小鼠自主食用甜菊糖苷后前尿液蛋白质组的共有差异蛋白，具有与脑奖励回路相关的蛋白。

通过非监督聚类分析，能将小鼠自主食用安赛蜜后前的总尿液蛋白质组进行区分，成组分析小鼠自主食用安赛蜜后前尿液蛋白质组差异蛋白及其富集的生物学过程，部分与糖脂代谢相关，也和有神经系统相关的生物学过程，差异蛋白中有和脑奖励回路相关的蛋白，但没有和甜味觉感知相关的蛋白；单只分析小鼠自主食用安赛蜜后前尿液蛋白质组的共有差异蛋白，具有与代谢和脑奖励回路相关的蛋白。

成组分析小鼠自主食用三氯蔗糖后前尿液蛋白质组差异蛋白及其富集的生物学过程，大部分与糖脂代谢相关，也和有神经系统相关的生物学过程，差异蛋白中有和脑奖励回路相关的蛋白，但是没有和甜味觉感知相关的蛋白；单只分析小鼠自主食用三氯蔗糖后前尿液蛋白质组的共有差异蛋白，具有与代谢和脑奖励回路相关的蛋白。

对 4 种甜味物质的横向比较结果进行总结。4 种甜味物质中，三氯蔗糖与蔗糖引起机体的变化最为相似，甜菊糖苷与蔗糖引起机体的变化相差最远；蔗糖、安赛蜜和三氯蔗糖引起的机体变化相似，而甜菊糖苷引起机体的变化与其他甜味物质不同。4 种甜味物质导致的尿液蛋白质组差异蛋白中出现和脑奖励回路相关的蛋白，在不同个体之间是相对稳定存在的。其中蔗糖、安赛蜜和三氯蔗糖导致的尿液蛋白质组差异蛋白中出现和糖脂代谢等相关的蛋白，在蔗糖组不同个体之间不是相对稳定存在的，而在安赛蜜和三氯蔗糖组不同个体之间不是相对稳定存在的。

具体如表 15 所示。

表 15 4 种甜味物质尿液蛋白质组差异蛋白的横向比较

	差异蛋白	生物学过程	分子功能	脑奖励回路	稳定性
Sucrose	65	主要与糖、脂代谢相关，也涉及到神经系统	涉及胰岛素受体激活	相关	代谢过程稳定性较差，脑奖励回路稳定性较强
Stevia Glycosides	66	主要与核小体组装、基因表达相关，也涉及到神经系统	不涉及胰岛素受体激活	相关	脑奖励回路稳定性较强
Acesulfame	93	主要与糖、脂代谢相关，也涉及到神经系统	不涉及胰岛素受体激活	相关	代谢过程和脑奖励回路稳定性较强
Sucralose	83	主要与糖、脂代谢相关，也涉及到神经系统	涉及胰岛素受体激活	相关	代谢过程和脑奖励回路稳定性较强

6 讨论

小鼠自主食用不同甜味物质后，尿液蛋白质组立刻发生了变化，反应十分迅速。且不同甜味物质导致的变化并不相同，变化与代谢、脑奖励回路等过程相关。以往不同甜味剂对机体的影响、顾客对不同甜味剂的喜好程度的研究，需要进行长期的、大样本量的食用和调查，需要投入大量的人力和物力，而通过尿液蛋白质进行研究，则可大大缩短研究时间和成本。本研究展示了尿液蛋白质组在研究各类甜味物质对机体影响的潜力，为探索开发更安全、更稳定和更令顾客喜爱的非营养性甜味剂提供了新的尿液蛋白质学研究方法。进一步实验可以考虑使用更多甜味物质、扩大实验动物样本量或收集人类样本进行研究；同时也反映出尿液蛋白组的灵敏性，为尿液蛋白质组功能的探索开辟新的领域。

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附表 1 4 种甜味物质成组分析的共有差异蛋白

Names	total	Uniprot ID	Protein name
Acesulfame stevia glycosides sucralose sucrose	1	Q61147	Ceruloplasmin
Acesulfame stevia glycosides sucrose	3	P57096	Prostate stem cell antigen
		P99029	Peroxiredoxin-5, mitochondrial
		Q80YX1	Tenascin (TN)
stevia glycosides sucralose sucrose	1	P03953	Complement factor D
acesulfame sucralose sucrose	1	P28665	Murinoglobulin-1
acesulfame stevia glycosides sucralose	3	P70441	Na(+)/H(+) exchange regulatory cofactor NHE-RF1
		Q60928	Glutathione hydrolase 1 proenzyme
		Q60823	RAC-beta serine/threonine-protein kinase
acesulfame sucrose	5	Q64729	TGF-beta receptor type-1
		Q9QWR8	Alpha-N-acetylgalactosaminidase
		Q7M6Z0	Reticulon-4 receptor-like 2
		P11152	Lipoprotein lipase (LPL)
		P16092	Fibroblast growth factor receptor 1
sucralose sucrose	11	P11087	Collagen alpha-1(I) chain
		A2BIM8	Major urinary protein 18
		Q9DAK9	14 kDa phosphohistidine phosphatase
		P12023	Amyloid-beta precursor protein
		Q01768	Nucleoside diphosphate kinase B
		P23953	Carboxylesterase 1C
		P63101	14-3-3 protein zeta/delta
		Q921I1	Serotransferrin
		P41272	CD27 antigen

		P04939	Major urinary protein 3
		Q9DBJ1	Phosphoglycerate mutase 1
acesulfame stevia glycosides	16	P48441	Alpha-L-iduronidase
		O35215	D-dopachrome decarboxylase
		P52760	2-iminobutanoate/2-iminopropanoate deaminase
		P13597	Intercellular adhesion molecule 1
		P51910	Apolipoprotein D
		Q9DAS9	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-12
		Q3UVY5	Pecanex-like protein 4
		Q61398	Procollagen C-endopeptidase enhancer 1
		O54965	E3 ubiquitin-protein ligase RNF13
		Q9R1E6	Ectonucleotide pyrophosphatase/phosphodiesterase family member 2
		P35505	Fumarylacetoacetase
		P61458	Pterin-4-alpha-carbinolamine dehydratase
		P10649	Glutathione S-transferase Mu 1
		Q08761	Vitamin K-dependent protein S
		Q9ES90	G-protein coupled receptor 35
		Q9DAU7	WAP four-disulfide core domain protein 2
stevia glycosides sucralose	6	P62737	Actin, aortic smooth muscle
		P51655	Glypican-4 (K-glypican)
		Q8VCS0	N-acetylmuramoyl-L-alanine amidase
		P19221	Prothrombin
		P14152	Malate dehydrogenase, cytoplasmic
		Q64726	Zinc-alpha-2-glycoprotein
acesulfame sucralose	6	Q91WV7	Amino acid transporter heavy chain SLC3A1
		P04441	H-2 class II histocompatibility antigen gamma chain
		P12658	Calbindin
		P61110	Kidney androgen-regulated protein
		Q91XE4	N-acyl-aromatic-L-amino acid amidohydrolase
		Q00724	Retinol-binding protein 4
sucrose	43	P11859	Angiotensinogen
		P11680	Properdin

		Q9R0M4	Podocalyxin
		P97384	Annexin A11
		P04940	Ig kappa chain V-VI region NQ2-17.4.1
		P11591	Major urinary protein 5
		Q99N23	Carbonic anhydrase 15
		Q3U962	Collagen alpha-2(V) chain
		Q5FW60	Major urinary protein 20
		P19001	Keratin, type I cytoskeletal 19
		P21614	Vitamin D-binding protein
		P97798	Neogenin
		P45700	Mannosyl-oligosaccharide 1,2-alpha-mannosidase IA
		P06797	Procathepsin L
		P13634	Carbonic anhydrase 1
		P31786	Acyl-CoA-binding protein
		Q8VCR7	Putative protein-lysine deacylase ABHD14B
		O09131	Glutathione S-transferase omega-1
		Q61391	Enkephalinase
		Q3UDR8	Protein YIPF3
		P07758	Alpha-1-antitrypsin 1-1
		P05533	Lymphocyte antigen 6A-2/6E-1
		Q9R0G6	Cartilage oligomeric matrix protein
		Q8R0I0	Angiotensin-converting enzyme 2
		Q8K209	Adhesion G-protein coupled receptor G1
		P11276	Fibronectin (FN)
		P97426	Eosinophil cationic protein 1
		Q8QZR4	Out at first protein homolog
		P06869	Urokinase-type plasminogen activator
		A1L317	Keratin, type I cytoskeletal 24
		Q8BND5	Sulfhydryl oxidase 1
		P13020	Gelsolin
		P26262	Plasma kallikrein
		P46412	Glutathione peroxidase 3
		E9PV24	Fibrinogen alpha chain
		O88322	Nidogen-2
		P49183	Deoxyribonuclease-1
		P10126	Elongation factor 1-alpha 1
		P16675	Lysosomal protective protein
		P06909	Complement factor H

		P00920	Carbonic anhydrase 2
		P35459	Lymphocyte antigen 6D
		Q00898	Alpha-1-antitrypsin 1-5
stevia glycosides	36	B1AZI6	THO complex subunit 2
		P62806	Histone H4
		P07141	Macrophage colony-stimulating factor 1
		Q01721	Growth arrest-specific protein 1
		Q921W8	Secreted and transmembrane protein 1A
		Q8K1H9	Odorant-binding protein 2a
		Q8C110	SLIT and NTRK-like protein 6
		P00687	Alpha-amylase 1
		Q9JI02	Secretoglobin family 2B member 20
		Q8BX35	Tumor necrosis factor receptor superfamily member 27
		Q9Z0M9	Interleukin-18-binding protein
		Q9D7R7	Gastricsin
		Q3V0K9	Plastin-1
		Q8R0W0	Epiplakin
		P02301	Histone H3.3C
		P97792	Coxsackievirus and adenovirus receptor homolog
		Q8BWP8	Beta-1,4-glucuronyltransferase 1
		P07361	Alpha-1-acid glycoprotein 2
		Q99PT1	Rho GDP-dissociation inhibitor 1
		Q9Z2J0	Solute carrier family 23 member 1
		O08709	Peroxiredoxin-6
		Q91WR6	Glycoprotein integral membrane protein 1
		Q64105	Sepiapterin reductase
		P15949	Kallikrein 1-related peptidase b9
		P11438	Lysosome-associated membrane glycoprotein 1
		Q7TSK7	ADAMTS-like protein 2
		P55292	Desmocollin-2
		Q3UMW8	Ceroid-lipofuscinosis neuronal protein 5 homolog
		P18181	CD48 antigen
		Q61088	Frizzled-4
		Q9JKR6	Hypoxia up-regulated protein 1
		Q9WVJ9	EGF-containing fibulin-like extracellular matrix protein 2

		Q6PGG6	Guanine nucleotide-binding protein-like 3-like protein
		P35441	Thrombospondin-1
		Q9Z138	Tyrosine-protein kinase transmembrane receptor ROR2
		Q03404	Trefoil factor 2
acesulfame	58	P01027	Complement C
		Q9ESD1	Prostasin
		Q8VEM8	Solute carrier family 25 member 3
		P26618	Platelet-derived growth factor receptor alpha
		Q91ZI0	Cadherin EGF LAG seven-pass G-type receptor 3
		Q60932	Voltage-dependent anion-selective channel protein 1
		P51885	Lumican
		Q91Y97	Fructose-bisphosphate aldolase B
		Q61247	Alpha-2-antiplasmin
		Q64449	C-type mannose receptor 2
		Q02819	Nucleobindin-1
		P35461	Lymphocyte antigen 6G
		P54763	Ephrin type-B receptor 2
		Q9R007	C-type lectin domain family 5 member A
		P26043	Radixin
		P55849	Desmocollin-1
		Q9CQW3	Vitamin K-dependent protein Z
		O09051	Guanylate cyclase activator 2B
		Q811B3	A disintegrin and metalloproteinase with thrombospondin motifs 12
		P31725	Protein S100-A9
		O89051	Integral membrane protein 2B
		Q14BI7	ATP-dependent RNA helicase TDRD9
		Q9JM99	Proteoglycan 4
		P07901	Heat shock protein HSP 90-alpha
		P18572	Basigin
		P08249	Malate dehydrogenase, mitochondrial
		Q9QXD6	Fructose-1,6-bisphosphatase 1
		Q9CZT5	Vasorin
		P35846	Folate receptor alpha
		Q9CR33	MANSC domain-containing protein

			1
		O09133	Submaxillary gland androgen-regulated protein 2, isoform alpha
		P70699	Lysosomal alpha-glucosidase
		P31809	Carcinoembryonic antigen-related cell adhesion molecule 1
		Q8VC60	Beta-galactosidase-1-like protein
		Q91V08	C-type lectin domain family 2 member D
		Q9DC11	Plexin domain-containing protein 2
		P20029	Endoplasmic reticulum chaperone BiP
		O08992	Syntenin-1
		Q9CQ60	6-phosphogluconolactonase
		P35802	Neuronal membrane glycoprotein M6-a
		P14094	Sodium/potassium-transporting ATPase subunit beta-1
		P48614	Protein Wnt-10b
		O35298	Acyloxyacyl hydrolase
		Q8C050	Ribosomal protein S6 kinase alpha-5
		Q6RKD8	Leucine-rich repeat transmembrane protein FLRT1
		Q61495	Desmoglein-1-alpha
		P20108	Thioredoxin-dependent peroxide reductase, mitochondrial
		P23780	Beta-galactosidase
		Q9D8B7	Junctional adhesion molecule C
		Q9JLT2	Trehalase
		Q05909	Receptor-type tyrosine-protein phosphatase gamma
		Q08423	Trefoil factor 1
		O35448	Lysosomal thioesterase PPT2
		P17563	Methanethiol oxidase
		O35305	Tumor necrosis factor receptor superfamily member 11A
		P81117	Nucleobindin-2
		Q9JHY4	WAP four-disulfide core domain protein 15B
		Q8K426	Resistin-like gamma
sucralose	54	P08905	Lysozyme C-2
		Q99P86	Resistin-like beta
		P05784	Keratin, type I cytoskeletal 18

		Q9DBH5	Vesicular integral-membrane protein VIP36
		P30275	Creatine kinase U-type, mitochondrial
		P62962	Profilin-1
		P11588	Major urinary protein 1
		O35188	Fractalkine
		P60710	Actin, cytoplasmic 1
		P08228	Superoxide dismutase
		O89017	Legumain
		Q9JJS0	Signal peptide, CUB and EGF-like domain-containing protein 2
		P10493	Nidogen-1
		B5X0G2	Major urinary protein 17
		P25119	Tumor necrosis factor receptor superfamily member 1B
		B2RPV6	Multimerin-1
		Q61592	Growth arrest-specific protein 6
		Q9JHY3	WAP four-disulfide core domain protein 12
		Q8R1U2	Cell growth regulator with EF hand domain protein 1
		P09411	Phosphoglycerate kinase 1
		Q9WTR5	Cadherin-13
		P97808	FXYD domain-containing ion transport regulator 5
		Q9Z319	Atrial natriuretic peptide-converting enzyme
		P17182	Alpha-enolase
		P16125	L-lactate dehydrogenase B chain
		P10923	Osteopontin
		D3YXG0	Hemicentin-1
		A2AEP0	Odorant-binding protein 1b
		Q923L3	CUB and sushi domain-containing protein 1
		P05064	Fructose-bisphosphate aldolase A
		P05201	Aspartate aminotransferase, cytoplasmic
		Q6PDN3	Myosin light chain kinase, smooth muscle
		P47878	Insulin-like growth factor-binding protein 3
		Q9ET66	Peptidase inhibitor 16

		P52480	Pyruvate kinase PKM
		O08810	116 kDa U5 small nuclear ribonucleoprotein component
		P56480	ATP synthase subunit beta, mitochondrial
		P55288	Cadherin-11
		P50543	Protein S100-A11
		P45376	Aldo-keto reductase family 1 member B1
		Q9R1P3	Proteasome subunit beta type-2
		P50516	V-type proton ATPase catalytic subunit A
		Q61090	Frizzled-7 (Fz-7) (mFz7)
		Q91WZ8	Dysbindin
		P63017	Heat shock cognate 71 kDa protein
		Q9ESB3	Histidine-rich glycoprotein
		O88307	Sortilin-related receptor
		Q02596	Glycosylation-dependent cell adhesion molecule 1
		Q9DCG2	CD302 antigen
		Q6ZWQ0	Nesprin-2
		P04186	Complement factor B
		O70250	Phosphoglycerate mutase 2
		P32261	Antithrombin-III
		P35700	Peroxiredoxin-1

附表 2 4 种甜味物质成组分析的共有生物学过程

Names	total	elements
acesulfame stevia glycosides sucralose sucrose	1	positive regulation of cell migration
acesulfame sucralose sucrose	3	cell adhesion
		blood coagulation
		negative regulation of peptidase activity
acesulfame sucrose	3	positive regulation of inflammatory response
		collagen fibril organization
		angiogenesis
sucralose sucrose	19	energy reserve metabolic process
		mitochondrion morphogenesis
		negative regulation of transcription, DNA-templated
		cellular response to lipid
		negative regulation of lipid storage

		locomotor rhythm
		response to bacterium
		positive regulation of lipid metabolic process
		positive regulation of MAPK cascade
		negative regulation of lipid biosynthetic process
		negative regulation of insulin secretion involved in cellular response to glucose stimulus
		aerobic respiration
		positive regulation of glucose metabolic process
		negative regulation of gluconeogenesis
		heat generation
		cellular response to testosterone stimulus
		positive regulation of protein kinase B signaling
		glucose homeostasis
		positive regulation of gene expression
acesulfame stevia glycosides	3	peripheral nervous system axon regeneration
		negative regulation of platelet-derived growth factor receptor signaling pathway
		positive regulation of cell proliferation
stevia glycosides sucralose	3	regulation of cell shape
		mesenchyme migration
		skeletal muscle thin filament assembly
acesulfame sucralose	4	gluconeogenesis
		negative regulation of apoptotic process
		carbohydrate metabolic process
		positive regulation of ERK1 and ERK2 cascade
sucrose	34	platelet aggregation
		regulation of cardiac conduction
		response to cytokine
		vascular smooth muscle cell differentiation
		response to chromate
		regulation of systemic arterial blood pressure by renin-angiotensin
		angiotensin-activated signaling pathway
		positive regulation of neuron projection development
		skin development
		fibrinolysis
		response to lead ion
		response to estrogen
		positive regulation of CoA-transferase activity
		regulation of gene expression
		ERK1 and ERK2 cascade
		kidney development

		plasminogen activation
		angiotensin-mediated drinking behavior
		mesenchymal cell differentiation
		skeletal system development
		cell-matrix adhesion
		extracellular matrix organization
		learning or memory
		one-carbon metabolic process
		proteolysis
		blood coagulation, fibrin clot formation
		response to peptide hormone
		response to methanol
		positive regulation of epithelial to mesenchymal transition
		complement activation, alternative pathway
		positive regulation of gap junction assembly
		regulation of cell adhesion
		negative regulation of endopeptidase activity
		negative regulation of serine-type endopeptidase activity
stevia glycosides	20	regulation of centromere complex assembly
		DNA-templated transcription, initiation
		negative regulation of plasma membrane long-chain fatty acid transport
		regulation of gene silencing
		DNA replication-independent nucleosome assembly
		cell proliferation
		tetrahydrobiopterin biosynthetic process
		nucleosome assembly
		Wnt signaling pathway, calcium modulating pathway
		DNA replication-dependent nucleosome assembly
		Wnt signaling pathway
		sensory perception of sound
		actin filament network formation
		actin filament bundle assembly
		pericentric heterochromatin assembly
		positive regulation of epithelial cell proliferation involved in wound healing
		regulation of gene expression, epigenetic
		negative regulation of chromosome condensation
		negative regulation of megakaryocyte differentiation
		L-phenylalanine metabolic process
acesulfame	20	cellular response to reactive oxygen species

		response to lipopolysaccharide
		regulation of ruffle assembly
		lipid metabolic process
		positive regulation of cellular extravasation
		positive regulation of NF-kappaB transcription factor activity
		animal organ morphogenesis
		metabolic process
		positive regulation of phospholipase C activity
		cell-cell adhesion
		palate development
		protein stabilization
		chondrocyte differentiation
		skeletal system morphogenesis
		positive regulation of peptidyl-tyrosine phosphorylation
		microvillus assembly
		positive regulation of nitric oxide biosynthetic process
		positive regulation of kinase activity
		protein localization to plasma membrane
		homophilic cell adhesion via plasma membrane adhesion molecules
sucralose	65	histone H4 acetylation
		positive regulation of binding
		synaptic vesicle endocytosis
		positive regulation of transcription, DNA-templated
		negative regulation of cell migration
		positive regulation of muscle contraction
		striated muscle contraction
		actin cytoskeleton organization
		morphogenesis of a polarized epithelium
		establishment of Golgi localization
		canonical glycolysis
		glycolytic process through fructose-6-phosphate
		methylglyoxal biosynthetic process
		positive regulation of protein phosphorylation
		ATP biosynthetic process
		retina development in camera-type eye
		glycolytic process
		cellular response to electrical stimulus
		glyceraldehyde-3-phosphate biosynthetic process
		histone H2A acetylation
		axonogenesis
		muscle cell cellular homeostasis

		postsynaptic actin cytoskeleton organization
		extrinsic apoptotic signaling pathway
		NAD metabolic process
		positive regulation of T cell differentiation
		regulation of protein localization to plasma membrane
		cellular phosphate ion homeostasis
		regulation of blood coagulation
		protein localization to adherens junction
		establishment or maintenance of cell polarity
		cell motility
		negative regulation of neuron death
		positive regulation of cell motility
		hemostasis
		cellular response to interleukin-7
		negative regulation of cyclin-dependent protein kinase activity
		metanephric collecting duct development
		adherens junction assembly
		protein homotetramerization
		platelet activation
		peripheral nervous system myelin maintenance
		negative regulation of protein binding
		cytolysis by host of symbiont cells
		positive regulation of phosphorylation
		ATP metabolic process
		aging
		positive regulation of double-strand break repair via homologous recombination
		negative regulation of cell growth
		negative regulation of hypoxia-induced intrinsic apoptotic signaling pathway
		regulation of transmembrane transporter activity
		Notch signaling pathway
		regulation of multicellular organism growth
		response to mechanical stimulus
		regulation of norepinephrine uptake
		cellular response to cytochalasin B
		cellular iron ion homeostasis
		memory
		positive regulation of plasminogen activation
		protein trimerization
		positive regulation of phagocytosis
		positive regulation of cytokine-mediated signaling

		pathway
		regulation of cyclin-dependent protein serine/threonine kinase activity
		response to heat
		apical protein localization

附表 3 4 种甜味物质成组分析的共有差异蛋白分子功能

Names	total	elements
acesulfame stevia glycosides sucralose sucrose	1	hydrolase activity
acesulfame sucralose sucrose	4	peptidase inhibitor activity
		identical protein binding
		receptor binding
		serine-type endopeptidase inhibitor activity
acesulfame stevia glycosides sucralose	1	calcium ion binding
acesulfame sucrose	1	endopeptidase inhibitor activity
sucralose sucrose	7	peptidase activity
		odorant binding
		protease binding
		small molecule binding
		pheromone binding
		extracellular matrix structural constituent
		insulin-activated receptor activity
acesulfame stevia glycosides	2	peroxiredoxin activity
		thioredoxin peroxidase activity
stevia glycosides sucralose	1	Wnt-protein binding
sucrose	12	endopeptidase activity
		heparin binding
		fibronectin binding
		vitamin D binding
		hydro-lyase activity
		heparan sulfate proteoglycan binding

		carbonate dehydratase activity
		peptidase activator activity
		arylesterase activity
		proteoglycan binding
		collagen binding
		acetylcholine receptor binding
stevia glycosides	7	integrin binding
		structural constituent of chromatin
		chromatin binding
		protein domain specific binding
		PDZ domain binding
		DNA binding
		protein heterodimerization activity
acesulfame	15	growth factor binding
		macromolecular complex binding
		carbohydrate binding
		protein binding involved in protein folding
		beta-galactosidase activity
		signaling receptor activity
		hydrolase activity, acting on glycosyl bonds
		protein tyrosine kinase activity
		beta-amyloid binding
		syndecan binding
		virus receptor activity
		hydrolase activity, hydrolyzing O-glycosyl compounds
		antioxidant activity
		transmembrane receptor protein tyrosine kinase activity
		protein homodimerization activity
sucralose	23	ADP binding
		intramolecular transferase activity, phosphotransferases
		serine-type peptidase activity
		structural constituent of postsynaptic actin cytoskeleton
		enzyme binding
		cadherin binding
		catalytic activity
		nitric-oxide synthase binding
		2,3-bisphosphoglycerate-dependent phosphoglycerate mutase activity
		nucleotide binding

		Tat protein binding
		heat shock protein binding
		ATP binding
		Wnt-activated receptor activity
		fructose binding
		fructose-bisphosphate aldolase activity
		bisphosphoglycerate mutase activity
		phosphopyruvate hydratase activity
		growth factor receptor binding
		chaperone binding
		kinesin binding
		protein binding
		phosphoglycerate mutase activity

附表 4 4 种甜味物质单个分析共有的共有差异蛋白

Names	total	Uniprot ID	Protein name
acesulfame stevia glycosides sucralose	1	Q60928	Glutathione hydrolase 1 proenzyme
acesulfame stevia glycosides	1	P52760	2-iminobutanoate/2-iminopropanoate deaminase
acesulfame sucralose	2	P70441	Na(+)/H(+) exchange regulatory cofactor NHE-RF1
		P12658	Calbindin
sucrose	1	Q61391	Enkephalinase
acesulfame	10	Q91ZI0	Cadherin EGF LAG seven-pass G-type receptor 3
		Q9JI02	Secretoglobin family 2B member 20
		Q60932	Voltage-dependent anion-selective channel protein 1
		P99029	Peroxiredoxin-5, mitochondrial
		Q9DAU7	WAP four-disulfide core domain protein 2
		Q91XE4	N-acyl-aromatic-L-amino acid amidohydrolase
		P23780	Beta-galactosidase
		P70699	Lysosomal alpha-glucosidase
		Q06318	Uteroglobin
		Q9JHY4	WAP four-disulfide core domain protein 15B
sucralose	8	P11087	Collagen alpha-1(I) chain
		P04939	Major urinary protein 3
		P15626	Glutathione S-transferase Mu 2
		P05784	Keratin, type I cytoskeletal 18
		Q02596	Glycosylation-dependent cell adhesion molecule 1
		P41272	CD27 antigen

		P10923	Osteopontin
		P09411	Phosphoglycerate kinase 1